

# The Chemokine (C-C Motif) Receptor 2 Antagonist INCB3284 Reduces Fluid Requirements and Protects From Hemodynamic Decompensation During Resuscitation From Hemorrhagic Shock

**OBJECTIVES:** Clinical correlations suggest that systemic chemokine (C-C motif) ligand (CCL) 2 release may contribute to blood pressure regulation and the development of hemodynamic instability during the early inflammatory response to traumatic-hemorrhagic shock. Thus, we investigated whether blockade of the principal CCL2 receptor chemokine (C-C motif) receptor (CCR) 2 affects blood pressure in normal animals, and hemodynamics and resuscitation fluid requirements in hemorrhagic shock models.

**DESIGN:** Randomized prospective treatment study.

**SETTING:** University laboratory.

**SUBJECTS:** Male Sprague-Dawley rats.

**INTERVENTIONS:** First, treatment of healthy anesthetized rats with increasing doses of INCB3284 or vehicle. Second, rats were hemorrhaged for 30 minutes, followed by treatment with the CCR2 antagonist INCB3284 (1.1 and 5.5  $\mu\text{mol/kg}$ ), the CCR5 antagonist Maraviroc (=control, 5.5  $\mu\text{mol/kg}$ ) or vehicle, and subsequent fluid resuscitation to maintain blood pressure until  $t = 90$  minutes. Third, treatment of rats with 5  $\mu\text{mol/kg}$  INCB3284 or vehicle after hemorrhage and fluid resuscitation until  $t = 300$  minutes.

**MEASUREMENTS AND MAIN RESULTS:** INCB3284 did not affect intrinsic function of isolated rat resistance arteries in pressure myography experiments. Blood pressure in anesthetized vehicle-treated animals continuously decreased by  $0.09 \pm 0.01$  mm Hg/min ( $p < 0.001$ ) but remained constant after INCB3284 injections. Systemic concentrations of the CCR2 agonists CCL2, CCL5, and CCL11 increased during hemorrhage and fluid resuscitation. INCB3284 dose-dependently reduced fluid requirements by  $58\% \pm 11\%$  in short-term experiments, whereas Maraviroc and vehicle-treated animals were indistinguishable. When resuscitation was performed until  $t = 300$  minutes, INCB3284 reduced fluid requirements by  $62\% \pm 6\%$ , prevented from hemodynamic decompensation, reduced mortality from 50% with vehicle treatment to zero, and reduced overall tissue wet-weight/dry-weight ratios.

**CONCLUSIONS:** Our findings suggest that CCR2 is involved in the regulation of normal cardiovascular function and during the cardiovascular stress response to hemorrhagic shock and fluid resuscitation. The present study identifies CCR2 as a drug target to reduce fluid requirements and to prevent death from hemodynamic decompensation during resuscitation from hemorrhagic shock.

**KEYWORDS:** chemokine (C-C motif) ligand 2; fluid requirements; hemodynamics; hemorrhage; shock; survival

Anthony J. DeSantis, MD<sup>1</sup>

McWayne Weche, MD<sup>1</sup>

Garrett A. Enten, MS<sup>1,2</sup>

Xianlong Gao, MD, PhD<sup>1</sup>

Matthias Majetschak, MD, PhD<sup>1,2</sup>

Copyright © 2022 The Authors. Published by Wolters Kluwer Health, Inc. on behalf of the Society of Critical Care Medicine. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

DOI: 10.1097/CCE.0000000000000701

**H**emorrhagic shock (HS) is the major cause of potentially preventable death after accidental injuries. HS accounts for over 35% of prehospital deaths and over 40% of deaths within the first 24 hours in trauma patients (1). Despite the urgent need to improve outcomes from HS, treatment options are limited. Pharmacological approaches to improve fluid resuscitation and reduce adverse effects associated with current treatment strategies, such as fluid overload, are not available. Such drugs, however, would likely have a significant clinical impact and could save the lives of many patients (1, 2).

It is known that tissue injury and hepatic hypoxia during traumatic-hemorrhagic shock (T/HS) drive inflammation, which contributes to vascular dysfunction, impaired endothelial barrier function, and hemodynamic instability. Chemokines, such as chemokine (C-C motif) ligand (CCL) 2 or CCL5, have been identified as key drivers that initiate and amplify the very early inflammatory response to T/HS and fluid resuscitation in animals and humans (3–7). Systemic prehospital CCL2 concentrations have been shown to be significantly increased in hypotensive trauma patients, compared with normotensive trauma patients, and systemic CCL2 concentrations within 24 hours of admission have been reported to segregate surviving from nonsurviving trauma patients (3, 8). Although the mechanisms underlying these important clinical correlations are poorly understood, they suggest that CCL2 release may contribute to blood pressure regulation and the development of hemodynamic instability during the early inflammatory response to T/HS.

CCL2 is the principal endogenous agonist of chemokine (C-C motif) receptor (CCR) 2 (9). CCR2 is expressed on various immune cells, on vascular endothelial and smooth muscle cells, and in multiple other organs and tissues (9). Although CCL2 is also an agonist at CCR3 and CCR5, the binding affinity of CCL2 for these receptors is more than 100-fold lower than the binding affinity of CCL2 for CCR2 ( $K_d \approx 0.5$  nM) (10–12).

Due to the important roles of chemokine receptors in numerous disease processes, various selective chemokine receptor antagonists have been developed, among which the CCR5 antagonist Maraviroc and the chemokine (C-X-C motif) receptor (CXCR) 4 antagonist AMD3100 are approved by the Federal Drug Administration (13–17). INCB3284 is a selective CCR2 antagonist, which reached phase IIa trials in patients with rheumatoid arthritis (13,

18). The effects of INCB3284 during resuscitation from HS, however, have not been evaluated. The aim of the present study was to determine whether blockade of the major CCL2 receptor CCR2 with the bona fide antagonist INCB3284 would affect hemodynamics and fluid requirements in HS and fluid-resuscitation models in rats.

## MATERIALS AND METHODS

### Drugs

INCB3284 and Maraviroc were purchased from Tocris (Minneapolis, MN). Phenylephrine was obtained from Millipore Sigma (St. Louis, MO).

### In Vivo Animal Experiments

All procedures were performed in accordance with the National Institutes of Health Guidelines for Use of Laboratory Animals and were approved by the University of South Florida Institutional Animal Care and Use Committee (IS00008139). Male Sprague-Dawley rats (300–400 g, Envigo, Indianapolis, IN) were anesthetized with 1.7% isoflurane via nose-cone inhalation with the SomnoSuite anesthesia system (Kent Scientific, Torrington, CT). At this dose, rats did not respond to noxious stimuli but maintained spontaneous respiration. The left femoral artery was isolated and cannulated with a 24-gauge peripheral IV catheter to allow for blood withdrawal, hemodynamic monitoring, drug administration, and fluid resuscitation. Hemodynamics were continuously monitored using the SurgivetV6400 blood pressure monitor (Med-Electronics, Beltsville, MD), and blood pressures and heart rate were recorded at 1–10-minute intervals. All animals were observed over a 10-minute period to ensure hemodynamic stability before start of the experiments. All experiments were performed randomized.

To test the effects of INCB3284 in normal rats, animals received repetitive injections of 1 mL of Lactated Ringer (LR) solution (vehicle) in 10-minute intervals ( $n = 3$ ) or increasing doses of the drug (0.55, 0.82, 1.4, and 2.75  $\mu\text{mol/kg}$  INCB3284) in 1 mL LR in 10 minute intervals ( $n = 3$ ).

To test the effects of INCB3284 after HS, we used a Wiggers fixed pressure hemorrhage and fluid-resuscitation model, as we described (19–23), with slight modifications. As an additional control drug, we selected the CCR5 antagonist Maraviroc. In brief, rats were hemorrhaged to a mean arterial blood pressure

(MAP) of less than or equal to 30 mm Hg for a period of 30 minutes. At the end of the HS period ( $t = 30$  min), animals were injected with either vehicle (1 mL LR) or drug in 1 mL LR, followed by fluid resuscitation with 1 mL bolus injections of LR to maintain a systolic blood pressure of 90 mm Hg or an MAP of 60 mm Hg. To avoid fluid overload, bolus injections of LR were limited to 1 mL/min. In the first series of experiments, animals received 1 mL of LR ( $n = 9$ ), 1.1  $\mu\text{mol/kg}$  ( $n = 4$ ) or 5.5  $\mu\text{mol/kg}$  ( $n = 5$ ) INCB3284 in 1 mL LR, or 5.5  $\mu\text{mol/kg}$  Maraviroc ( $n = 3$ ) in 1 mL LR at  $t = 30$  minutes. Animals were then resuscitated until  $t = 90$  minutes. At  $t = 0, 15, 30, 60,$  and 90 minutes, blood samples were obtained, and plasma was prepared. Samples were stored at  $-70^\circ\text{C}$  until further processing. At  $t = 90$  minutes, animals were euthanized (isoflurane inhalation and bilateral pneumothorax). Dosing of INCB3284 in normal animals and in the first series of HS was chosen based on the dosing of INCB3284 that was previously used to define its pharmacokinetic profile in rats, dogs, and primates (13).

In the second series of experiments, animals received 1 mL of LR ( $n = 10$ ) or 5  $\mu\text{mol/kg}$  INCB3284 in 1 mL LR ( $n = 8$ ) at  $t = 30$  minutes. The dosing of INCB3284 was chosen based on the results of the first series of experiments. Animals were resuscitated until  $t = 300$  minutes to simulate a clinically relevant duration of fluid resuscitation. Blood samples were obtained for arterial blood gas analyses and measurements of routine laboratory parameters at time points  $t = 0, 30, 90, 150, 210, 270,$  and 300 minutes. Death was defined as asystole or loss of a pulse pressure. At the end of the experiments, surviving animals were euthanized as described before. In animals that survived until  $t = 300$  minutes, a gross necropsy was performed after euthanasia, and tissue from lung, small intestine, and colon was collected for measurements of wet-weight/dry-weight ratios.

### Pressure Myography

Because we previously proposed that CCR antagonists should be screened for cross-reactivity with  $\alpha_1$ -adrenoceptors to exclude potential adverse cardiovascular effects when used in vivo (24), we employed pressure myography to exclude that INCB3284 would affect function of isolated rat resistance arteries and vasoconstriction induced by the selective  $\alpha_1$ -adrenoceptor agonist phenylephrine. Pressure myography with rat

mesenteric arteries was performed as described in detail previously (23–27). In brief, after euthanasia, the mesentery was removed, and third- or fourth-order mesenteric arteries were dissected free from the mesentery, mounted onto two glass cannulas, and pressurized to 60 mm Hg in a DMT110P pressure myograph (DMT-USA, Ann Arbor, MI). The vessel bath solution was continuously aerated with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$  throughout the experiment. The outer diameter of the pressurized vessel was then continuously measured and recorded via digital video-edge detection. INCB3284 (10  $\mu\text{M}$ ) or vehicle was added to the vessel bath. After 15 minutes, increasing doses of phenylephrine were added in 10-minute intervals.

### Arterial Blood Gases and Routine Laboratory Parameters

Arterial blood gases, electrolytes, creatinine, lactate, hematocrit, and hemoglobin were analyzed using the Element point of care veterinary blood gas, electrolyte, and critical care analyzer (Cuattro Veterinary USA, Loveland, CO).

### Protein Measurements

Total protein concentrations in plasma were determined on a Nanodrop 1000 (Thermo Scientific, Ashville, NC). Bovine serum albumin served as protein standard. Due to variable degrees of hemolysis in the recovered plasma, sample absorption at  $\lambda = 413$  nm was measured, and the hemoglobin concentration in the sample was calculated according to (28) and subtracted from the total protein concentration.

### Measurements of Chemokine Concentrations

To assess whether cognate agonists of CCR2, CCR3, and CCR5 are systemically released after HS and fluid resuscitation in rats under our experimental conditions, we measured plasma levels of CCL2 (CCR2/3/5 agonist), CCL5 (CCR1/3/5 agonist), CCL7 (CCR1/2/3 agonist), CCL11 (CCR2/3/5 agonist), and chemokine (C-X-C motif) ligand (CXCL) 10 (CCR3 and CXCR3 agonist) in vehicle-treated animals (9). Chemokine concentrations in plasma were measured with commercially available enzyme-linked immunosorbent assays (ELISA) according to the manufacturers' instructions. ELISA kits for CCL2 and CCL5 were purchased from R&D systems (Minneapolis, MN) and

for CCL7, CCL11, and CXCL10 from MyBioSource (San Diego, CA). Chemokine concentrations were expressed per mg of total protein corrected for hemoglobin to account for dilutional effects due to continuous fluid resuscitation.

### Wet-Weight/Dry-Weight Ratios

The ratio of the tissue wet weight to dry weight was determined gravimetrically, as previously described (29–32).

### Data Analyses and Statistics

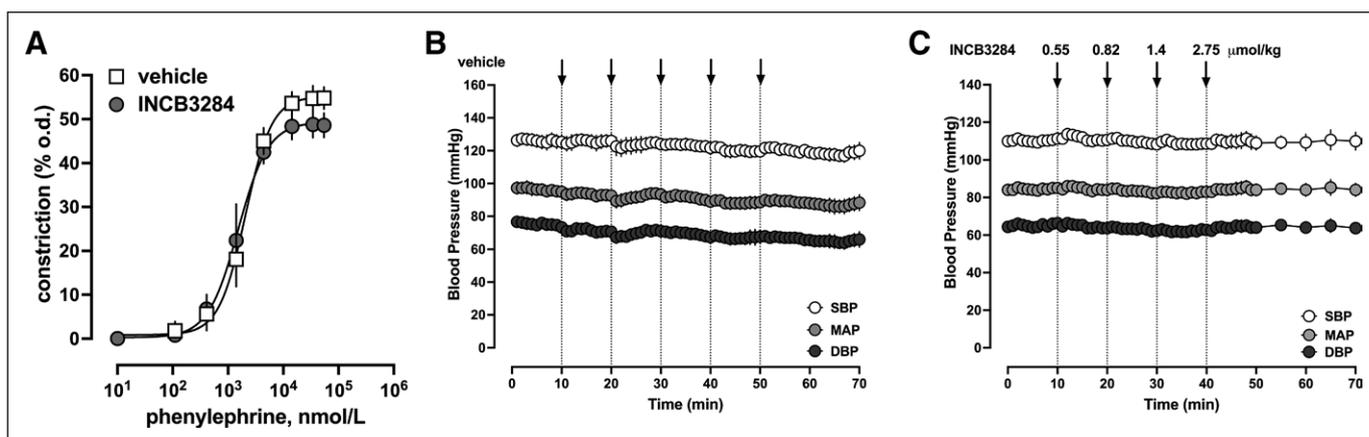
Data are presented as mean  $\pm$  SE or median with interquartile range (25th/75th percentile). Data were analyzed by Student *t* test, 1-way analysis of variance (ANOVA), or 2-way ANOVA with Dunnett multiple comparisons tests, as appropriate. Survival curves were analyzed using the log-rank test. Proportions were compared with the Fisher exact test. Blood pressure trends and dose-responses were analyzed with linear and nonlinear regression analyses, respectively. All data analyses were calculated with the GraphPad Prism program, Version 9.3.1 (GraphPad Software, San Diego, CA). A two-tailed  $p < 0.05$  was considered significant.

## RESULTS

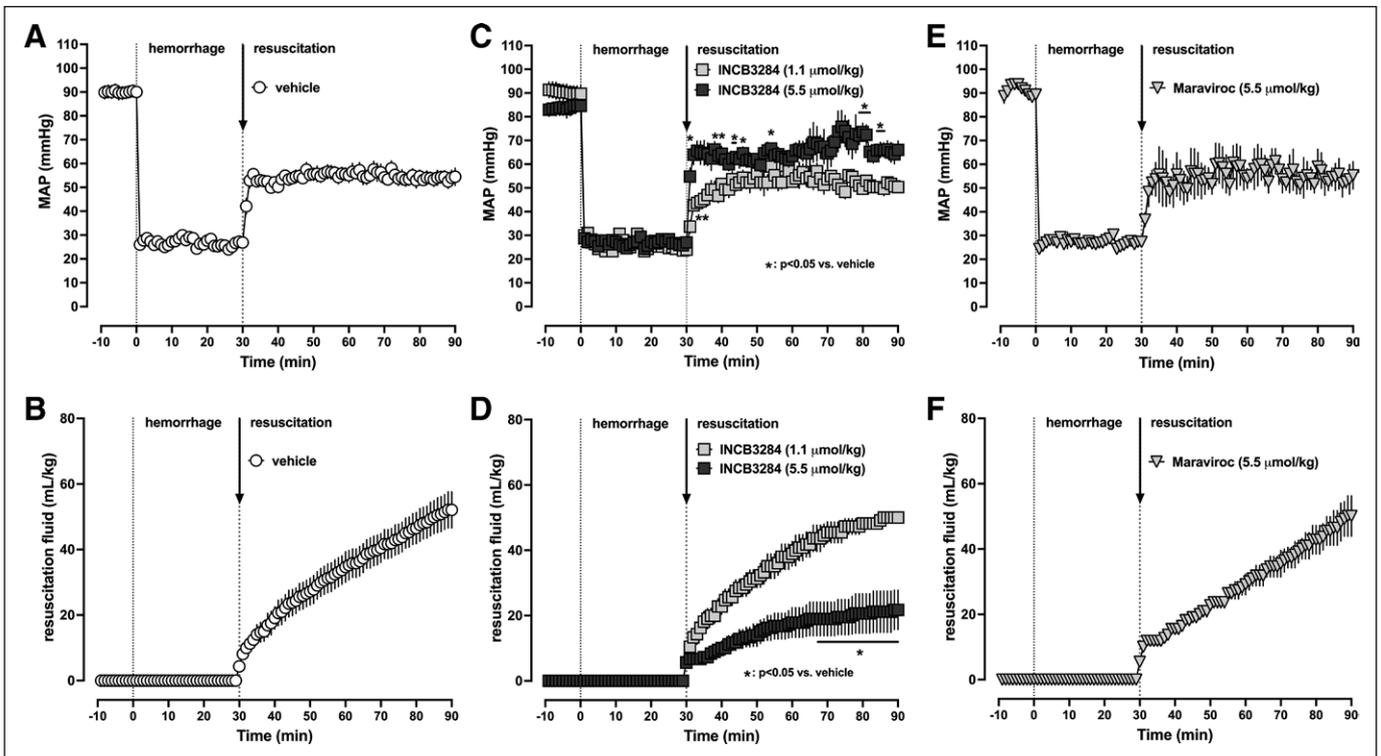
As compared with arteries preexposed to vehicle, preexposure of arteries to 10  $\mu$ M INCB3284 did not alter

artery diameter or affect phenylephrine-induced vasoconstriction (**Fig. 1A**). Furthermore, we tested whether injection of INCB3284 would affect blood pressure in normal rats. **Figure 1, B and C**, show the blood pressures in animals receiving repetitive bolus injections of 1 mL of vehicle and INCB3284, respectively. Linear regression analysis showed that MAP in vehicle-treated animals continuously decreased by  $0.09 \pm 0.01$  mm Hg/min (95% CI, 0.1–0.07 mm Hg/min;  $r^2$ , 0.25;  $p < 0.001$ ; Fig. 1B). In contrast, MAP in animals injected with increasing doses of INCB3284 in a total volume of 1 mL remained constant during the observation period (Fig. 1C). The slope of the MAP linear regression line in animals treated with INCB3284 was  $-0.011$  mm Hg/min (95% CI,  $-0.04$  to  $0.016$  mm Hg/min;  $r^2$ , 0.004;  $p = 0.43$ ).

Next, we tested whether administration of a single bolus injection of INCB3284 would affect hemodynamics and fluid requirements in a short-term model of HS, when compared with vehicle- and Maraviroc-treated animals. All animals were indistinguishable at baseline. To achieve an MAP of 30 mm Hg during the HS period, vehicle-treated animals were hemorrhaged to  $49.7 \pm 1.9\%$  total blood volume (**Supplemental Digital Content 1A**, <http://links.lww.com/CCX/A996>). Target blood pressures during fluid resuscitation could be achieved in all vehicle-treated animals. Fluid requirements to maintain target blood pressures averaged to  $52.1 \pm 5.5$  mL/kg after vehicle treatment (**Fig. 2, A and B**).



**Figure 1.** Effects of INCB3284 on intrinsic vascular function and on blood pressure in normal rats. **A**, Pressure myography with isolated rat mesenteric arteries. Arteries were exposed to vehicle or 10  $\mu$ M of INCB3284 for 15 min, followed by increasing doses of phenylephrine. Constriction (% o.d.) represents constriction in percent of the outer diameter of the artery at baseline before exposure to vehicle or INCB3284. Data are mean  $\pm$  SE,  $n = 4$  from four different animals per condition. Animals received repetitive injections of 1 mL vehicle (Lactated Ringer's solution; **B**) or increasing doses of INCB3284 in 1 mL vehicle (**C**). Blood pressures are provided in mm Hg. Arrows indicate time points of vehicle or drug injection. Data are mean  $\pm$  SE,  $n = 3$ /group. DBP = diastolic blood pressure, MAP = mean arterial blood pressure, SBP = systolic blood pressure.



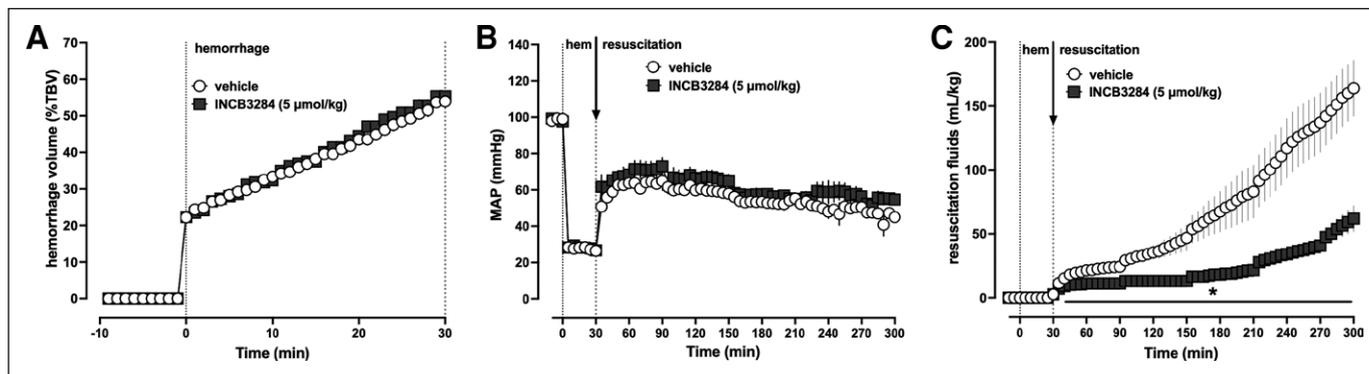
**Figure 2.** Fluid requirements to maintain hemodynamics after hemorrhagic shock. All data are mean  $\pm$  SE. Arrows indicate time points of drug injection. **A** and **B**, Animals were treated with vehicle. **C** and **D**, Animals were treated with 1.1  $\mu\text{mol/kg}$  (light gray squares,  $n = 4$ ) and 5.5  $\mu\text{mol/kg}$  INCB3284 (dark gray squares,  $n = 5$ ). **E** and **F**, Animals were treated with 5.5  $\mu\text{mol/kg}$  Maraviroc ( $n = 3$ ). **A**, **C**, and **E**, Mean arterial blood pressure (MAP; mm Hg). **B**, **D**, and **F**, Fluid requirements (mL/kg) to achieve an MAP of 60 mm Hg or a systolic blood pressure of 90 mm Hg. \* $p < 0.05$  versus vehicle-treated animals.

All vehicle-treated animals survived the resuscitation period. Although CCL7 was not detectable, systemic concentrations of CCL2, CCL5, and CCL11 per mg of total protein significantly increased during fluid resuscitation from HS (**Supplemental Digital Content 2A–D**, <http://links.lww.com/CCX/A996>).

**Figure 2C–F** show the effects of the chemokine receptor antagonists when administered at the beginning of fluid resuscitation. The hemorrhage volumes to produce the target MAP during the shock period were comparable among all groups ( $p > 0.05$  vs vehicle-treated animals; **Supplemental Digital Content 1, B and C**, <http://links.lww.com/CCX/A996>). All animals could be resuscitated with crystalloids to the target blood pressures and survived the observation period. Although administration of 1.1  $\mu\text{mol/kg}$  of INCB3284 did not affect fluid requirements to maintain hemodynamics ( $50 \pm 1$  mL/kg;  $p > 0.05$  vs vehicle), 5.5  $\mu\text{mol/kg}$  INCB3284 reduced fluid requirements by  $58\% \pm 11\%$  ( $21.7 \pm 6$  mL/kg;  $p < 0.05$  vs vehicle and 1.1  $\mu\text{mol/kg}$  INCB3284) and increased MAP at various time points during the observation period (Fig. 2, C and D). Administration of 5.5  $\mu\text{mol/kg}$  of Maraviroc did not

affect fluid requirements, when compared with vehicle-treated animals or with animals treated with 1.1  $\mu\text{mol/kg}$  of INCB3284 (Fig. 2, E and F).

To assess whether these fluid-sparing effects of INCB3284 could have therapeutic potential, we tested whether a single dose of 5  $\mu\text{mol/kg}$  of drug would have beneficial effects over a clinically more relevant observation period of 300 minutes (**Fig. 3**). Hemorrhage volumes to achieve a MAP of 30 mm Hg were comparable among vehicle-treated and INCB3284-treated animals (**Fig. 3A**). Vehicle-treated animals required on average  $163 \pm 22$  mL/kg of resuscitation fluid (**Fig. 3, B and C**). Target blood pressures during fluid resuscitation could be maintained in only two of the 10 vehicle-treated animals throughout the observation period. With INCB3284 treatment, target blood pressures during fluid resuscitation could be maintained in all animals ( $p < 0.05$  vs vehicle-treated animals), and average fluid requirements were reduced by  $62\% \pm 6\%$  ( $61.9 \pm 10$  mL/kg;  $p < 0.05$ ). **Figure 4, A and B**, shows the fluid requirements for all individual animals treated with vehicle and INCB3284, respectively. Vehicle-treated animals that did not survive the observation



**Figure 3.** INCB3284 reduces fluid requirements during resuscitation from hemorrhagic shock. All data are mean  $\pm$  SE. Arrows indicate time points of vehicle/drug injection. Open circles indicate vehicle-treated animals ( $n = 10$ ). Dark squares indicate INCB3284 (5  $\mu$ mol/kg)-treated animals ( $n = 8$ ). \* $p < 0.05$  versus vehicle-treated animals. **A**, Hemorrhage volumes to achieve a mean arterial blood pressure (MAP) of 30 mm Hg. %TBV represents percent total blood volume. **B**, MAP (mm Hg). **C**, Fluid requirements to achieve an MAP of 60 mm Hg or a systolic blood pressure of 90 mm Hg.

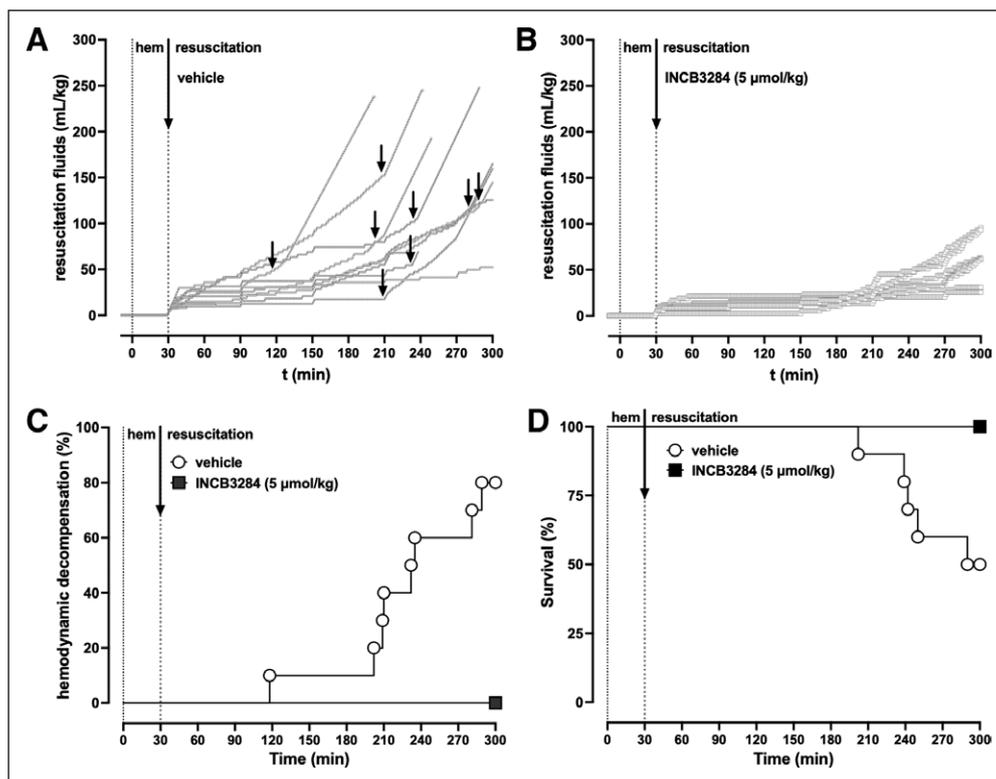
period showed a steep increase in fluid requirements preceding cardiovascular collapse (Fig. 4A; marked with arrows). We considered this turning point of the fluid requirements as the beginning of hemodynamic

decompensation. Eight of the 10 vehicle-treated animals reached the beginning of hemodynamic decompensation. The median time to the beginning of hemodynamic decompensation was 233.5 minutes with

vehicle treatment (Fig. 4C).

Three vehicle-treated animals that reached this turning point after  $t = 210$  minutes survived until  $t = 300$  minutes. None of the INCB3284-treated animals demonstrated the beginning of hemodynamic decompensation (Fig. 4, B and C;  $p < 0.01$  versus vehicle-treated animals).

Hematocrit values were indistinguishable between the groups (Supplemental Digital Content 3A, <http://links.lww.com/CCX/A996>). Lactate concentrations increased to approximately 10 mmol/L at the end of the HS period ( $p > 0.05$  between groups) and partially normalized in vehicle- and INCB3284-treated animals (Supplemental Digital Content 3B, <http://links.lww.com/CCX/A996>).  $P_{CO_2}/P_{O_2}$  in arterial blood



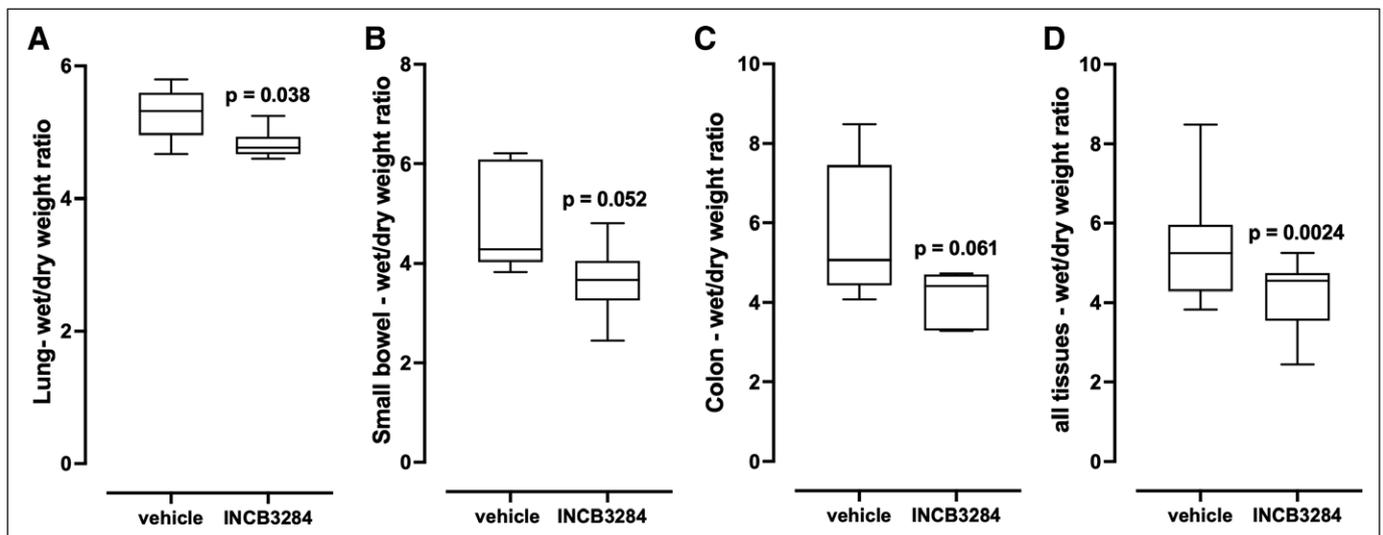
**Figure 4.** INCB3284 prevents hemodynamic decompensation during resuscitation from hemorrhagic shock. All data are mean  $\pm$  SE. Arrows indicate time points of vehicle/drug. **A**, Fluid requirements to achieve a mean arterial blood pressure (MAP) of 60 mm Hg or a systolic blood pressure (SBP) of 90 mm Hg of all individual vehicle-treated animals. Short arrows indicate the time point when fluid-resuscitation requirements to maintain target blood pressures increased, which we defined as beginning of hemodynamic decompensation. **B**, Fluid requirements to achieve an MAP of 60 mm Hg or an SBP of 90 mm Hg of all individual INCB3284-treated animals. **C**, Time to the beginning of hemodynamic decompensation. **D**, Kaplan-Meier survival curve.

and creatinine concentrations were indistinguishable between the groups (**Supplemental Digital Content 3C–E**, <http://links.lww.com/CCX/A996>). Mortality was 50% in vehicle-treated animals and 0% in INCB3284-treated animals ( $p < 0.05$ ; **Fig. 4D**). Median survival time was 295 minutes with vehicle treatment and more than 300 minutes with INCB3284 treatment ( $p < 0.05$  vs vehicle). In animals that survived until  $t = 300$  minutes, lung wet-weight/dry-weight ratios were significantly reduced with INCB3284 treatment (**Fig. 5A**). Although differences between vehicle- and INCB3284-treated animals in small bowel and colon wet-weight/dry-weight ratios did not reach statistical significance individually (**Fig. 5, B and C**), wet-weight/dry-weight ratios of all tissues combined were significantly reduced with INCB3284 treatment (**Fig. 5D**).

## DISCUSSION

Consistent with our previous observations on the effects of INCB3284 on recombinant  $\alpha_{1b}$ -adrenergic receptors and with a reported safety pharmacology profile of this drug that permitted oral use in humans (13, 18, 24), we observed that INCB3284 has no direct effects on isolated rat resistance arteries, that it does not interfere with  $\alpha_1$ -adrenergic receptor-mediated vasoconstriction *ex vivo*, and that it has no acute hyper- or hypotensive effects in normal rats after systemic bolus injections. Furthermore, we detected that a cumulative dose of 5.52  $\mu\text{mol/kg}$  INCB3284 injected over 30 minutes prevented the slight but linear decrease

in blood pressure that was noticeable after repetitive vehicle injections in anesthetized rats under baseline conditions. The noticeable downward blood pressure drift with vehicle treatment implies that perianesthetic fluid requirements exceeded the fluid volume of 5 mL that was injected over 70 minutes. As CCL2 is known to be constitutively expressed in the systemic circulation (33, 34) and we confirmed that CCR2 agonists are systemically detectable in rats under our experimental baseline conditions, these findings further support a role of CCR2 in blood pressure regulation. In agreement with previous observations in rodents and humans, we confirmed that systemic concentrations of CCL2 and other chemokines that share chemokine receptors with CCL2 increase very early during fluid resuscitation from HS (3–7). INCB3284 and Maraviroc are potent and selective antagonists, which compete with chemokine binding to the corresponding receptors and inhibit receptor function with  $\text{IC}_{50}$  in the low nanomolar range (13–16). These very similar pharmacological characteristics justify the administration of the drugs at comparable concentrations in the present study. Although the effects of INCB3284 have not been tested in context of HS and fluid resuscitation, Maraviroc has previously been described to provide lung and liver protection in trauma-hemorrhage models in rats (35, 36). Information on the effects of Maraviroc on hemodynamics and fluid requirements, however, has not been provided in these studies. While animals treated with 5.5  $\mu\text{mol/kg}$  Maraviroc were indistinguishable from



**Figure 5.** INCB3284 reduces tissue wet-weight/dry-weight ratios after resuscitation from hemorrhagic shock. Wet-weight/dry-weight ratios of lung (**A**), small bowel (**B**), colon (**C**), and all tissues combined (**D**). Boxes extend from the 25th–75th percentile, and the horizontal line shows the median. Error bars show the range of data (minimum/maximum). The level of statistical significance is indicated.

vehicle-treated animals, INCB3284 dose-dependently reduced fluid-resuscitation requirements and stabilized blood pressure in short-term experiments. These effects, in combination with the observed systemic release of endogenous CCR2 agonists, suggest that activation of CCR2 plays a role in the regulation of the early cardiovascular stress response to HS and fluid resuscitation. Furthermore, we detected that a single dose of INCB3284 resulted in profound fluid-sparing effects over a resuscitation period 4.5 hours and prevented development of hemodynamic decompensation. We noted, however, that fluid-resuscitation requirements of INCB3284-treated animals increased over the final 30–60 minutes of the observation period. Given that the half-life of INCB3284 in rats is  $168 \pm 12$  minutes (13), it appears possible that optimized dosing regimens, such as administration of a second dose of drug at later time points, could further reduce fluid requirements. The finding that hematocrit values were indistinguishable between groups suggests that intravascular volume status was comparable. Furthermore, our finding that overall tissue wet-weight/dry-weight ratios were reduced with INCB3284 treatment implies that CCR2 blockade attenuates impairment of endothelial barrier function and development of HS-induced capillary leakage. Thus, these data point toward reduction of fluid shifts into tissues as one mechanism by which INCB3284 reduces resuscitation fluid requirements. Despite the reduced fluid requirements, lactate levels partially normalized with vehicle and INCB3284 treatment, and creatinine concentrations were indistinguishable between the groups. This suggests that the fluid-sparing effects of INCB3284 do not occur at the expense of tissue hypoperfusion, altered metabolism, or impairment of kidney function (37).

Although mortality was 50% with vehicle treatment, INCB3284 treatment reduced mortality to zero. Because blood gases and hematocrit were comparable among all animals, hypoxia or hemodynamic decompensation due to fluid overload with subsequent right heart failure unlikely accounts for mortality in this model. In combination with the steep increase in fluid requirements preceding cardiovascular collapse that we observed in vehicle-treated animals, irreversible shock with circulatory failure due to loss of vascular tone most likely accounts for mortality in our model. The latter is characteristic for cardiovascular collapse during HS and fluid resuscitation (38).

There are also several limitations of the present study. The sample sizes in each group were relatively small, and the median survival time in INCB3284-treated animals remains undefined. Therefore, the finding that INCB3284 treatment reduced mortality to 0% at  $t = 300$  minutes may or may not be associated with a clinically meaningful reduction in mortality at later time points. Furthermore, it should be noted that the present study was performed exclusively with male animals. Thus, long-term resuscitation studies in animals of both sexes will be required to refine the effects of INCB3284 on survival and to exclude sex differences in response to INCB3284 treatment in the future. Although we did not observe adverse effects of intravascular INCB3284 administration in the present study, INCB3284 has previously been evaluated as an orally administered drug in clinical trials, and information on the effects of this drug after intravascular injection in humans is not available (13, 18). Thus, we are unable to comment on the potential safety profile of INCB3284 when used as an injectable drug during fluid resuscitation from T/HS in humans.

## CONCLUSIONS

Our findings suggest that CCR2 is involved in the regulation of cardiovascular function in normal animals and during the cardiovascular stress response to HS and fluid resuscitation. The present study identifies CCR2 as a promising drug target that could be used to reduce fluid requirements and to prevent death from hemodynamic decompensation during resuscitation from HS. Although the mechanisms underlying beneficial effects of INCB3284 remain to be determined, our observations justify further exploration of the therapeutic potential and possible side effect profile of INCB3284 and other CCR2-targeting drugs in disease models that are associated with loss of vascular tone and cardiovascular collapse, such as T/HS, septic shock, or cardiac surgery with cardiopulmonary bypass (1, 39–42).

1 Department of Surgery, Morsani College of Medicine, University of South Florida, Tampa, FL.

2 Department of Molecular Pharmacology and Physiology, Morsani College of Medicine, University of South Florida, Tampa, FL.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the

HTML and PDF versions of this article on the journal's website (<http://journals.lww.com/ccejournal>).

Parts of this research have been presented at the 43<sup>rd</sup> Conference on Shock, Virtual Meeting, 2021.

Based on the results of this article, University of South Florida has filed a provisional patent application. Drs. DeSantis, Gao, and Majetschak are inventors. Research reported in this publication was supported by the National Institutes of Health under award number R01GM139811.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

For information regarding this article, E-mail: [majetschak@usf.edu](mailto:majetschak@usf.edu)

## REFERENCES

- Kauvar DS, Lefering R, Wade CE: Impact of hemorrhage on trauma outcome: An overview of epidemiology, clinical presentations, and therapeutic considerations. *J Trauma* 2006; 60:S3–11
- Heron M, Hoyert DL, Murphy SL, et al: Deaths: Final data for 2006. *Natl Vital Stat Rep* 2009; 57:1–134
- Ziraldo C, Vodovotz Y, Namas RA, et al: Central role for MCP-1/CCL2 in injury-induced inflammation revealed by *in vitro*, *in silico*, and clinical studies. *PLoS One* 2013; 8:e79804
- Almahmoud K, Namas RA, Abdul-Malak O, et al: Impact of injury severity on dynamic inflammation networks following blunt trauma. *Shock* 2015; 44:101–109
- Hsieh CH, Frink M, Hsieh YC, et al: The role of MIP-1 alpha in the development of systemic inflammatory response and organ injury following trauma hemorrhage. *J Immunol* 2008; 181:2806–2812
- Makley AT, Goodman MD, Friend LA, et al: Resuscitation with fresh whole blood ameliorates the inflammatory response after hemorrhagic shock. *J Trauma* 2010; 68:305–311
- Richter JR, Sutton JM, Belizaire RM, et al: Macrophage-derived chemokine (CCL22) is a novel mediator of lung inflammation following hemorrhage and resuscitation. *Shock* 2014; 42:525–531
- Almahmoud K, Namas RA, Zaaqoq AM, et al: Prehospital hypotension is associated with altered inflammation dynamics and worse outcomes following blunt trauma in humans. *Crit Care Med* 2015; 43:1395–1404
- Alexander SP, Christopoulos A, Davenport AP, et al; CGTP Collaborators: The concise guide to pharmacology 2017/18: G protein-coupled receptors. *Br J Pharmacol* 2017; 174(Suppl 1):S17–S129
- Napier C, Sale H, Mosley M, et al: Molecular cloning and radioligand binding characterization of the chemokine receptor CCR5 from rhesus macaque and human. *Biochem Pharmacol* 2005; 71:163–172
- Daugherty BL, Siciliano SJ, DeMartino JA, et al: Cloning, expression, and characterization of the human eosinophil eotaxin receptor. *J Exp Med* 1996; 183:2349–2354
- Coulin F, Power CA, Alouani S, et al: Characterisation of macrophage inflammatory protein-5/human CC cytokine-2, a member of the macrophage-inflammatory-protein family of chemokines. *Eur J Biochem* 1997; 248:507–515
- Xue CB, Feng H, Cao G, et al: Discovery of INCB3284, a potent, selective, and orally bioavailable hCCR2 antagonist. *ACS Med Chem Lett* 2011; 2:450–454
- White JR, Lee JM, Dede K, et al: Identification of potent, selective non-peptide CC chemokine receptor-3 antagonist that inhibits eotaxin-, eotaxin-2-, and monocyte chemoattractant protein-4-induced eosinophil migration. *J Biol Chem* 2000; 275:36626–36631
- Lai WY, Mueller A: Latest update on chemokine receptors as therapeutic targets. *Biochem Soc Trans* 2021; 49:1385–1395
- Woollard SM, Kanmogne GD: Maraviroc: A review of its use in HIV infection and beyond. *Drug Des Devel Ther* 2015; 9:5447–5468
- De Clercq E: Mozobil® (Plerixafor, AMD3100), 10 years after its approval by the US food and drug administration. *Antivir Chem Chemother* 2019; 27:2040206619829382
- Mackay CR: Moving targets: Cell migration inhibitors as new anti-inflammatory therapies. *Nat Immunol* 2008; 9:988–998
- Babu FS, LaPorte HM, Nassoioy SP, et al: Chemokine (C-X-C motif) receptor 4 regulates lung endothelial barrier permeability during resuscitation from hemorrhagic shock. *Physiol Res* 2019; 68:675–679
- Nassoioy SP, Babu FS, LaPorte HM, et al: Effects of the Kv7 voltage-activated potassium channel inhibitor linopirdine in rat models of haemorrhagic shock. *Clin Exp Pharmacol Physiol* 2018; 45:916–926
- Nassoioy SP, Byron KL, Majetschak M: Kv7 voltage-activated potassium channel inhibitors reduce fluid resuscitation requirements after hemorrhagic shock in rats. *J Biomed Sci* 2017; 24:8
- Bach HH 4th, Laporte HM, Wong YM, et al: Proteasome inhibition prolongs survival during lethal hemorrhagic shock in rats. *J Trauma Acute Care Surg* 2013; 74:499–507
- Bach HH 4th, Wong YM, Tripathi A, et al: Chemokine (C-X-C motif) receptor 4 and atypical chemokine receptor 3 regulate vascular  $\alpha_1$ -adrenergic receptor function. *Mol Med* 2014; 20:435–447
- DeSantis AJ, Enten GA, Gao X, et al: Chemokine receptor antagonists with alpha1-adrenergic receptor blocker activity. *J Basic Clin Physiol Pharmacol* 2021 Jun 21. [online ahead of print]
- Albee LJ, Eby JM, Tripathi A, et al:  $\alpha_1$ -Adrenergic receptors function within hetero-oligomeric complexes with atypical chemokine receptor 3 and chemokine (C-X-C motif) receptor 4 in vascular smooth muscle cells. *J Am Heart Assoc* 2017; 6:e006575
- Tripathi A, Vana PG, Chavan TS, et al: Heteromerization of chemokine (C-X-C motif) receptor 4 with  $\alpha_1A/B$ -adrenergic receptors controls  $\alpha_1$ -adrenergic receptor function. *Proc Natl Acad Sci U S A* 2015; 112:E1659–E1668
- Albee LJ, LaPorte HM, Gao X, et al: Identification and functional characterization of arginine vasopressin receptor 1A: Atypical chemokine receptor 3 heteromers in vascular smooth muscle. *Open Biol* 2018; 8:170207
- Pluim D, Jacobs BA, Krähenbühl MD, et al: Correction of peripheral blood mononuclear cell cytosolic protein for hemoglobin contamination. *Anal Bioanal Chem* 2013; 405:2391–2395
- Nassoioy SP, Babu FS, LaPorte HM, et al: Pharmacological modulation of C-X-C motif chemokine receptor 4 influences development

- of acute respiratory distress syndrome after lung ischaemia-reperfusion injury. *Clin Exp Pharmacol Physiol* 2018; 45:16–26
30. Garcia-Covarrubias L, Manning EW 3<sup>rd</sup>, Sorell LT, et al: Ubiquitin enhances the Th2 cytokine response and attenuates ischemia-reperfusion injury in the lung. *Crit Care Med* 2008; 36:979–982
  31. Geng Q, Romero J, Saini V, et al: A subset of 26S proteasomes is activated at critically low ATP concentrations and contributes to myocardial injury during cold ischemia. *Biochem Biophys Res Commun* 2009; 390:1136–1141
  32. Baker TA, Romero J, Bach HH 4<sup>th</sup>, et al: Effects of exogenous ubiquitin in a polytrauma model with blunt chest trauma. *Crit Care Med* 2012; 40:2376–2384
  33. Antonelli A, Fallahi P, Ferrari SM, et al: High serum levels of CXCL10 and CCL2 chemokines in untreated essential hypertension. *Int J Immunopathol Pharmacol* 2012; 25:387–395
  34. Komiyama M, Takanabe R, Ono K, et al: Association between monocyte chemoattractant protein-1 and blood pressure in smokers. *J Int Med Res* 2018; 46:965–974
  35. Liu FC, Zheng CW, Yu HP: Maraviroc-mediated lung protection following trauma-hemorrhagic shock. *Biomed Res Int* 2016; 2016:5302069
  36. Liu FC, Tsai YF, Yu HP: Maraviroc attenuates trauma-hemorrhage-induced hepatic injury through PPAR gamma-dependent pathway in rats. *PLoS One* 2013; 8:e78861
  37. Levitt DG, Levitt JE, Levitt MD: Quantitative assessment of blood lactate in shock: Measure of hypoxia or beneficial energy source. *Biomed Res Int* 2020; 2020:2608318
  38. Gómez H, Mesquida J, Hermus L, et al: Physiologic responses to severe hemorrhagic shock and the genesis of cardiovascular collapse: Can irreversibility be anticipated? *J Surg Res* 2012; 178:358–369
  39. Hoen S, Mazoit JX, Asehnoune K, et al: Hydrocortisone increases the sensitivity to alpha1-adrenoceptor stimulation in humans following hemorrhagic shock. *Crit Care Med* 2005; 33:2737–2743
  40. Shanmugam G: Vasoplegic syndrome—the role of methylene blue. *Eur J Cardiothorac Surg* 2005; 28:705–710
  41. Fischer GW, Levin MA: Vasoplegia during cardiac surgery: Current concepts and management. *Semin Thorac Cardiovasc Surg* 2010; 22:140–144
  42. Groeben H, Böttiger BW, Schäfer M, et al: [Catecholamine-resistant hypotension – an update]. *Anesthesiol Intensivmed Notfallmed Schmerzther* 2005; 40:412–418