A Selective V_{1A} Receptor Agonist, Selepressin, Is Superior to Arginine Vasopressin and to Norepinephrine in Ovine Septic Shock*

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Objective: Selective vasopressin V_{1A} receptor agonists may have advantages over arginine vasopressin in the treatment of septic shock. We compared the effects of selepressin, a selective V_{1A} receptor agonist, arginine vasopressin, and norepinephrine on hemodynamics, organ function, and survival in an ovine septic shock model.

Design: Randomized animal study.

Setting: University hospital animal research laboratory.

Subjects: Forty-six adult female sheep.

Interventions: Fecal peritonitis was induced in the anesthetized, mechanically ventilated, fluid-resuscitated sheep, and they were randomized in two successive phases. Three late-intervention groups (each $n = 6$) received IV selepressin (1 pmol/kg/min), arginine vasopressin (0.25 pmol [0.1 mU]/kg/min), or norepinephrine (3 nmol [0.5 μg]/kg/min) when mean arterial pressure remained less than 70 mm Hg despite fluid challenge; study drugs were thereafter titrated to keep mean arterial pressure at 70–80 mm Hg. Three early-intervention groups (each $n = 7$) received selepressin, arginine vasopressin, or norepinephrine at the same initial infusion rates as for the late intervention, but already when mean arterial pressure had decreased by 10% from baseline; doses were then titrated as for the late intervention. A control group ($n = 7$) received saline. All animals were observed until death or for a maximum of 30 hours.

Measurements and Main Results: In addition to hemodynamic and organ function assessment, plasma interleukin-6 and nitrite/nitrate levels were measured. In the late-intervention groups, selepressin delayed the decrease in mean arterial pressure and was associated with lower lung wet/dry weight ratios than in the other two groups. In the early-intervention groups, selepressin maintained mean arterial pressure and cardiac index better than arginine vasopressin or norepinephrine, slowed the increase in blood lactate levels, and was associated with less lung edema, lower cumulative fluid balance, and lower interleukin-6 and nitrite/nitrate levels. Selepressin-treated animals survived longer than the other animals.

Conclusions: In this clinically relevant model, selepressin, a selective V_{1A} receptor agonist, was superior to arginine vasopressin and to norepinephrine in the treatment of septic shock, especially when administered early. (*Crit Care Med* 2016; 44:23–31)

***See also p. 234.**

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Dr. He conducted the experiment, acquired and analyzed the data, and drafted the article. Dr. Su designed and directed the protocol. Dr. Taccone helped perform the experiments, interpret the data, and draft the article. Drs. Laporte, Kjølbye, and Reinheimer participated in the protocol design and critically revised the article. Dr. Zhang carried out the biochemical measurements. Drs. Xie and Moussa helped perform the experiments and acquire the data. Dr. Vincent was involved in the study design and in critically revising the article.

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Septic shock, defined as sepsis with persistent arterial hypotension despite adequate fluid resuscitation, affects about 15% of ICU patients and is an important cause of morbidity and mortality (1). Optimal vasopressor support represents an important aspect in the management of patients with septic shock (2). Arginine vasopressin (AVP) may have a place in the treatment of septic shock when administered in addition to norepinephrine (NE) (2). The addition of AVP may exert beneficial effects directly or by reducing the need for catecholamines, thus limiting their adverse effects, including immune suppression, stimulation of cellular metabolism, and myocardial injury (3). Some recent studies have suggested that AVP administration may also be of benefit in less severe forms of septic shock or as an early intervention to limit edema formation and organ damage, rather than as rescue therapy (4–7), but the effects of this approach on outcomes have not been fully investigated.

AVP stimulates several types of receptors (V_{1A}, V_{1B}, V_2, oxytocin receptors) and thus has effects other than just V_{1A} receptor-mediated vasopressor actions (8, 9). The agonist potency of AVP at the V_{1A} and V_2 receptors is of the same magnitude (i.e., AVP is a mixed V_{1A}/V_2 receptor agonist) (8, 10, 11). Because V_2 and oxytocin receptor stimulation may aggravate sepsis-induced vasodilation and V_2 receptor stimulation may promote fluid accumulation and increase the risk of microvascular thrombosis (12, 13), it is interesting to ask whether a selective V_{1A} receptor agonist would be preferable. In animal models of pneumonia-induced sepsis and septic shock, selective V_{1A} receptor agonists have been reported to reverse hypotension more effectively than other vasopressors (14, 15) and also to reduce vascular/capillary leakage (16), improve heart, lung, and kidney function, and prolong survival (14, 15).

We have developed a clinically relevant ovine model of peritonitis-induced septic shock with fever, tachycardia, hyperdynamic state, decreased vascular tone, early capillary leakage, and multiple organ failure, which has been used to test the effects of new therapeutic interventions (17, 18). In the present study, the effects on hemodynamics, oxygen consumption, organ function, and survival time of a selective V_{1A} receptor agonist, selepressin (FE 202158) (10, 11), were compared with those of AVP and NE when administered early and later in the course of sepsis. We hypothesized that selepressin would have more favorable effects on these variables than AVP and NE, especially when administered early. Some of the results of the current study have been previously reported in the form of an abstract (19).

METHODS

The study was approved by our institutional review board for animals. Care and handling of the animals were in accordance with National and International Guidelines (20).

We studied 46 healthy female sheep weighing 25–35 kg, premedicated with intramuscular midazolam (Dormicum; Roche SA, Brussels, Belgium) (0.25 mg/kg) and ketamine hydrochloride (Imalgine; Merial, Lyon, France) (20 mg/kg). After IV injection of fentanyl (Fentanyl; Janssen, Berchem, Belgium) (30 μg/kg) and rocuronium (Esmeron; Organon, Oss, The Netherlands) (0.1 mg/kg) and endotracheal intubation, mechanical ventilation was started in volume-controlled mode (Servo ventilator 300 C; Siemens, Upplands Väsby, Sweden) with a tidal volume of 10 mL/kg, a positive end-expiratory pressure (PEEP) of 5 cm H_2O, an inspiratory time/expiratory time of 1:2, and a square wave pattern. The stomach was emptied with an orogastric tube and a balloon catheter (14F; Teleflex Medical, Research Triangle Park, NC) was inserted into the bladder for collecting urine. Under aseptic conditions, a PiCCO arterial catheter (4F; Pulsion Medical Systems, Wayne, NJ) was placed in the right femoral vein. A venous introducer was placed in the right femoral artery and used to insert a 7F pulmonary artery catheter (Edwards Lifesciences, Irvine, CA). The catheters were connected to pressure transducers (Edwards Lifesciences). Through a midline laparotomy, ultrasonic Doppler flow probes were placed around the superior mesenteric artery and the left renal artery and the cecum was punctured to collect feces. After collection of 1.5 g/kg body weight of feces, the puncture site was disinfected and closed. A plastic tube was inserted through the abdominal wall into the abdominal cavity, and the abdomen was closed.

During the experiment, anesthesia was maintained with a continuous IV infusion of a mixture of ketamine (10 mg/kg/hr), morphine (0.5 mg/kg/hr), midazolam (0.5 mg/kg/hr), and rocuronium (0.1 mg/kg/hr). Ringer's lactate (RL) solution and 6% hydroxyethyl starch (HES) solution (Voluven; Fresenius Kabi, Schelle, Belgium) were initially infused at a rate of 2 mL/kg/hr each. Fluid resuscitation was titrated to prevent hypovolemia, indicated by a stable pulmonary arterial occlusion pressure (PAOP) during the study period. When mean arterial pressure (MAP) remained less than 70 mm Hg despite fluid boluses (100 mL RL plus 100 mL HES given over 6 min), the fluid infusion was gradually reduced to 2 mL/kg/hr to avoid rapid development of refractory respiratory failure. Hypokalemia (< 3.5 mEq/L) and hypoglycemia (< 40 mg/dL) were corrected during the experiment.

Experimental Protocol

After baseline measurements, 1.5 g/kg/body weight feces were injected into the abdominal cavity to induce peritonitis, and the animals were then randomized into different groups (see below). Vasopressors were continuously infused via the cephalic vein as used for clinical administration. The doses of vasopressors were selected on the basis of previous experience from exploratory research in sheep (10). Animals were observed until death or for a maximum of 30 hours, after which they were killed by potassium chloride injection.

This study was performed in two successive phases (**Fig. 1**): late intervention, to compare the effects of selepressin, AVP,

and NE in established shock; and early intervention, to compare the effects of selepressin, AVP, and NE early in the development of shock.

Late Intervention. The study vasopressor was administered when MAP remained less than 70 mm Hg despite fluid challenge (defined as "refractory hypotension") and titrated to target an MAP of 70–80 mm Hg (target MAP). Each titration step was maintained for at least 15 minutes to determine whether the infusion rate was of sufficient magnitude to meet the requirements of the animal. Animals were randomized into three groups (each $n = 6$).

- Selepressin ([Phe^2,Ile^3,Hgn^4,$Orn(iPr)^8$]vasopressin; Ferring Pharmaceuticals A/S, Copenhagen, Demark) infusion was started at a dose of 1 pmol/kg/min and increased stepwise by 1 pmol/kg/min to a maximum of 10 pmol/kg/min to maintain the target MAP.
- AVP (American Regent, Shirley, NY) infusion was started at a dose of 0.25 pmol/kg/min (0.1 mU/kg/min) and increased stepwise by 0.25 pmol/kg/min to a maximum of 2.5 pmol/kg/min (1 mU/kg/min) to maintain the target MAP.
- NE (Levophed; Hospira Benelux BVBA, Brussels, Belgium) infusion was started at a dose of 3 nmol/kg/min (0.5 μg/kg/min) and increased stepwise by 3 nmol/kg/min to a maximum of 30 nmol/kg/min (5 μg/kg/min) to maintain the target MAP.

Early Intervention. The study vasopressor was infused if MAP decreased by 10% from baseline. The initial infusion rate was fixed until MAP decreased to less than 70 mm Hg despite fluid challenge. Thereafter, the vasopressor doses were titrated to maintain MAP at 70–80 mm Hg as in the previous experiments, each titration step being maintained for at least 15 minutes. Animals were randomized into three groups (each $n = 7$).

- Selepressin: animals received an initial dose of 1 pmol/kg/min selepressin; when MAP decreased less than 70 mm Hg despite fluid challenge, selepressin doses were increased stepwise by 1 pmol/kg/min to a maximum of 10 pmol/kg/min to maintain target MAP.
- AVP: animals received an initial dose of 0.25 pmol/kg/min (0.1 mU/kg/min); when MAP decreased less than 70 mm Hg despite fluid challenge, vasopressin doses were then increased stepwise by 0.25 pmol/kg/min to a maximum of 2.5 pmol/kg/min (1 mU/kg/min) to maintain MAP.
- NE: animals received an initial dose of 3 nmol/kg/min (0.5 μg/kg/min); when MAP decreased less than 70 mm Hg despite fluid challenge, NE doses were increased stepwise by 3 nmol/kg/min to a maximum of 30 nmol/kg/min (5 μg/kg/min) to maintain target MAP.

A control group of seven animals received 0.9% saline solution with no vasopressors.

Measurements

Heart rate, MAP, and mean pulmonary artery pressure (MPAP) (Sirecust 404; Siemens, Erlangen, Germany), cardiac output and core temperature (Vigilance monitor; Edwards Lifesciences), renal and superior mesenteric arterial blood flow (T208 flowmeter; Transonic Systems, Ithaca, NY), and ventilator parameters were monitored continuously; right atrial pressure, PAOP, arterial and mixed-venous blood gases, hemoglobin concentration, blood lactate, and electrolyte concentrations (ABL725 and OSM3; Radiometer Medical A/S, Brønshøj, Denmark) were measured hourly; urine output and infused volume were all recorded hourly. The time to develop oliguria (defined as urine < 0.5 mL/kg/hr) was also recorded. Extravascular lung water (EVLW) (PiCCO; Pulsion Medical Systems) was determined every 2 hours. Arterial plasma and urine were collected at baseline and at 6, 12, 18, 24, and 30 hours after feces injection and sent to the central laboratory department in Erasme Hospital, Brussels, Belgium, for biochemical measurements, including protein, creatinine, coagulation parameters, and colloid oncotic pressure. A set of plasma samples was stored at –70° before shipping to the Bioanalytical Laboratory of Ferring Pharmaceuticals A/S for measurement of selepressin concentration.

Derived variables, such as cardiac index, stroke volume index, systemic and pulmonary vascular resistance index, left ventricular stroke work index (LVSWI), oxygen delivery index (Do_2I), and oxygen consumption index (Vo_2I) were calculated using standard formulas as in previous studies (18). Dynamic lung compliance was calculated as tidal volume/(peak inspiratory pressure – PEEP) (21). The cumulative fluid balance was estimated as the difference between cumulative infusion volume and cumulative urine output.

When the animal died, the bloodless wet weight of the central lobe of the right lung was determined. Its dry weight was also measured after the lobe had been dehydrated for 24 hours in an oven at 200°C. The wet/dry weight ratio was used as an estimate of pulmonary edema severity.

Interleukin-6 and Nitrite Measurements

Plasma interleukin (IL)-6 was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) assay. Ninety-six well plates were coated with mouse antiovine IL-6 monoclonal antibody (MCA 1659; Serotec, Oxford, United Kingdom) diluted to 1 μg/mL and incubated at 4°C overnight. After aspirating the coating solution, blocking solution was added to the wells for 2 hours and then the coated plates were washed three times. Diluted samples were transferred into wells to incubate for 1 hour at 37°C, and then the plates were washed three times. Diluted rabbit antiovine IL-6 polyclonal antibody (AHP424; Serotec) was added to incubate for 1 hour at room temperature. The contents of each well were discarded and the plate rinsed three times; the substrate for sheep anti-rabbit immunoglobulin G:horseradish peroxidase (STAR54; Serotec) was then added to the plate and allowed to react for color development for 10 minutes. The optical density of the plate wells was then read on an ELISA plate reader at 450 nm.

Nitrite levels, as an index of nitric oxide (NO) concentration (22), were measured with a commercial kit (Parameter Total NO/Nitrite/ Nitrate, SKGE001; R&D Systems, Minneapolis, MN).

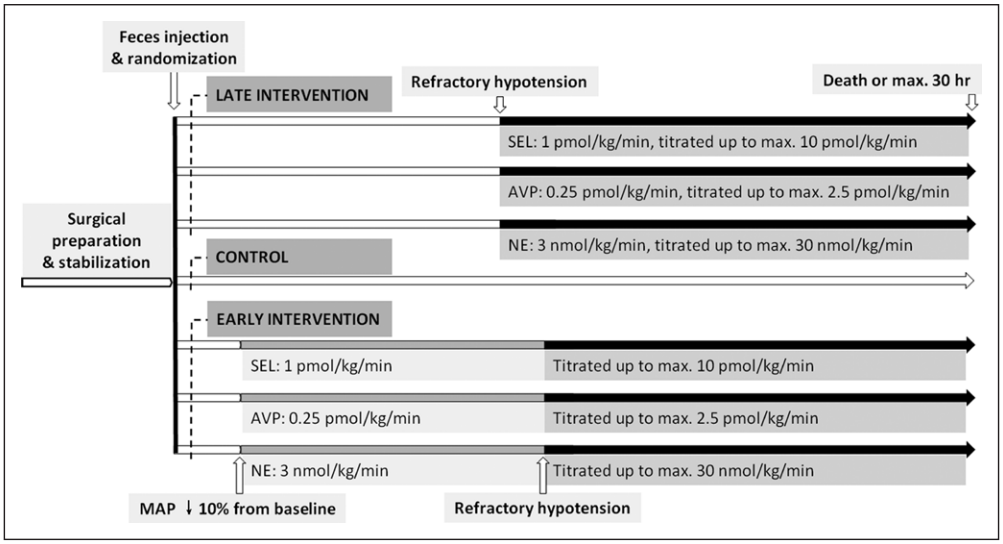

Figure 1. Schematic diagram of the experimental protocol. SEL = selepressin, AVP= arginine vasopressin, NE = norepinephrine, MAP = mean arterial pressure.

Statistical Analysis

Data were checked for normality using the Kolmogorov-Smirnov test. As no deviation from normality was detected, data are described as mean ± SD. Data were analyzed using linear mixed models with group, time, and group × time interactions as fixed effects, subjects (sheep) as a random effect, and vasopressor dose as a covariate. Each time point difference between selepressin and other groups was compared with a least significant difference adjustment in case of a significant group effect and/or of a significant time × group interaction. One-way analysis of variance with repeated measures followed by a least significant difference post hoc multiple comparisons was used to compare the time to develop oliguria, the time to develop a 10% decrease in MAP or hypotension, and the lung wet/dry ratio. Plasma selepressin concentration and survival times are expressed as median with interquartile ranges (25th–75th). Kaplan-Meier survival curves were constructed and analyzed using the log-rank test. Data were analyzed using IBM SPSS Statistics 19 for Windows (IBM, Somers, NY). All reported p values are two-sided, and a p value of less than 0.05 was considered to be statistically significant.

TABLE 1. Time to Develop Refractory Hypotension[a]

Group	Onset of Hypotension (Hr)	After Vasopressor Titration (Hr)
Control	11.3 ± 1.0[b]	Not applicable
Late intervention		
SEL	11.0 ± 1.8	12.9 ± 2.7
AVP	11.5 ± 0.9	14.3 ± 2.2
NE	11.2 ± 0.7	14.1 ± 2.6
Early intervention		
SEL	16.1 ± 2.3	19.0 ± 3.6
AVP	13.1 ± 1.3[b]	14.6 ± 1.4[b]
NE	12.1 ± 1.5[b]	13.7 ± 1.5[b]

SEL = selepressin, AVP = arginine vasopressin, NE = norepinephrine.
[a]Defined as mean arterial pressure < 70 mm Hg despite fluid challenge.
[b]$p < 0.05$ compared with early selepressin.

RESULTS

Late Intervention (Comparing the Effects of Selepressin, AVP, and NE in Established Shock)

Systemic Hemodynamics. After induction of peritonitis, all sheep developed fever, tachycardia, and hypotension with an increased cardiac output and decreased systemic vascular resistance (SVR). The initial time to develop refractory hypotension was similar in the three vasopressor groups and the control group (**Table 1**). Titration of the three vasopressors delayed the decrease in MAP (**Fig. 2*A***) and prolonged the time to develop hypotension similarly (Table 1), but the increases in heart rate and MPAP (**Table S1**, Supplemental Digital Content 1, http://links.lww.com/CCM/B479) were less marked in the selepressin group than in the NE group. There were no significant differences in cardiac index, SVR index (SVRI), or LVSWI among the groups (Fig. 2*A*; and Table S1, Supplemental Digital Content 1, http://links.lww.com/CCM/B479).

Organ Function and Regional Perfusion. Despite similar evolutions in the Pa_{O_2}/F_{IO_2} ratio, lung compliance, pH, and extravascular lung water index (EVLWI) in all groups (Table S1, Supplemental Digital Content 1, http://links.lww.com/CCM/B479), selepressin-treated animals had a lower postmortem lung wet/dry ratio than the other animals (**Fig. 3*A***). There was no significant difference in the cumulative fluid balance (**Fig. S1*A***, Supplemental Digital Content 1, http://links.lww.com/CCM/B479), $D_{O_2}I$, mixed venous oxygen saturation (Sv_{O_2}) (Table S1, Supplemental Digital Content 1, http://links.lww.com/CCM/B479), or blood lactate level (Fig. 2*A*). Renal blood flow, mesenteric blood flow, urine output, creatinine clearance, and time to develop oliguria (Table S1 and **Figs.** S1*A* and **S2*A***, Supplemental Digital Content 1, http://links.lww.com/CCM/B479) were similar in the treated groups.

Biological Mediators and Plasma Selepressin Concentration. Plasma IL-6 and plasma nitrite/nitrate concentrations increased in all groups (**Fig. 4*A***). Plasma IL-6 concentration increased less in the selepressin-treated animal group than in the NE and control groups. Plasma nitrite/nitrate concentrations increased similarly in the selepressin group and the control group, but less than in the NE group. Plasma selepressin

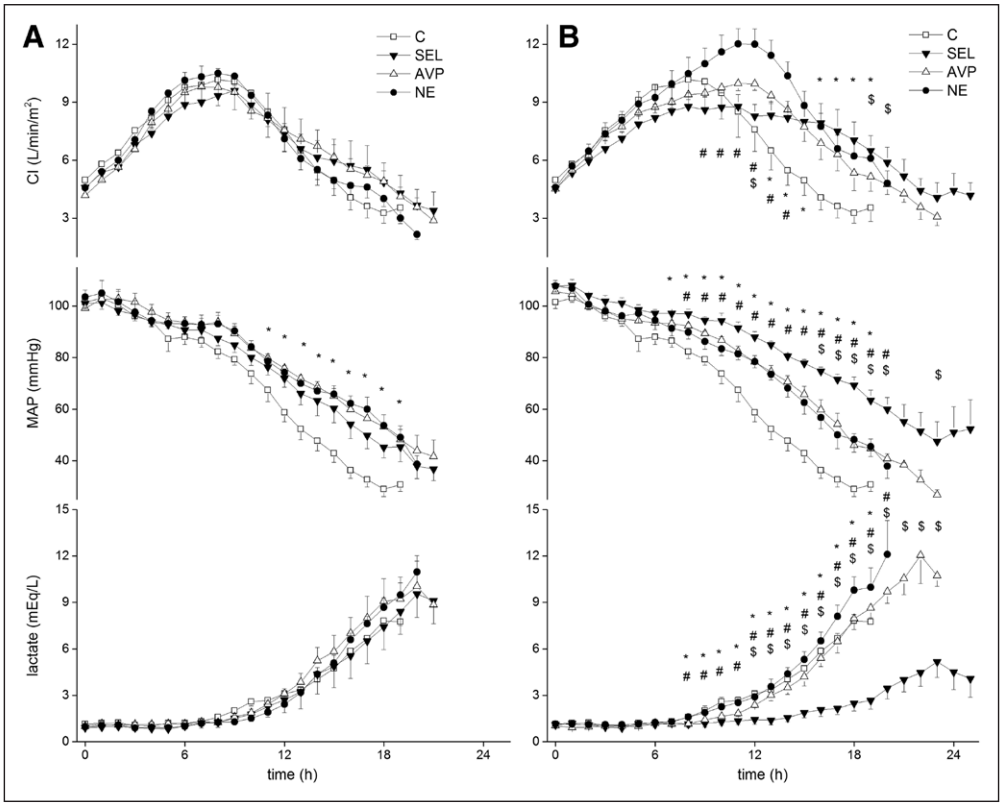

Figure 2. Evolution of cardiac index (CI), mean arterial pressure (MAP), and blood lactate concentration in late-intervention group (**A**) and early-intervention group (**B**). Time effects were significant. For the late intervention, group effects were nonsignificant except for MAP ($p < 0.05$), and group × time interaction effects were nonsignificant except for MAP. For the early intervention, group and group × time interaction effects were significant. */$/#$p < 0.05$ control group (C), arginine vasopressin (AVP), or norepinephrine (NE) compared with selepressin (SEL), respectively.

concentrations were 0.19 nmol/L (0.19–2.28) and 4.04 nmol/L (0.62–9.79) at 12 and 18 hours after initiation of selepressin infusion, respectively.

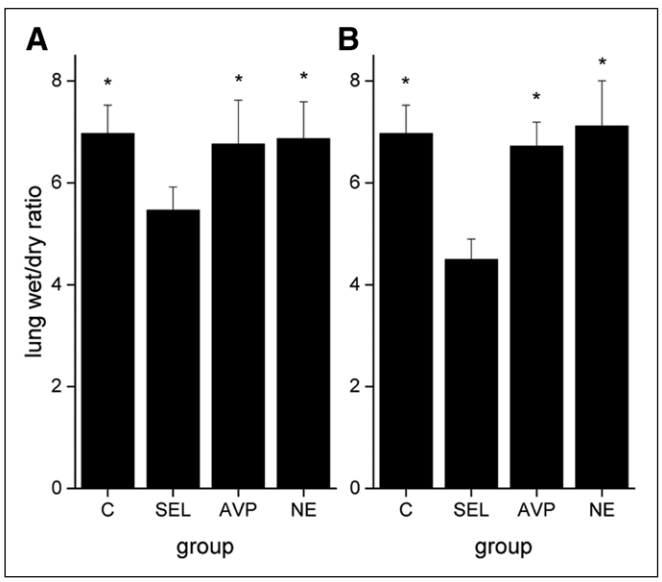

Figure 3. Postmortem lung wet/dry weight ratio in late-intervention group (**A**) and early-intervention group (**B**). The differences in late or early intervention were significant ($p < 0.005$). *$p < 0.005$ compared with selepressin (SEL). C = control group, AVP = arginine vasopressin, NE = norepinephrine.

Coagulation. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) increased progressively in all groups and fibrinogen concentration and platelet counts decreased (**Table S2**, Supplemental Digital Content 1, http://links.lww.com/CCM/B479). There were no significant differences in these parameters among groups.

Outcome. The median survival time in the selepressin group (21.0 hr [21.0–23.0]) was significantly longer than in the control (19.0 hr [18.5–21.0]) and NE (20.0 hr [20.0–20.5]) groups but similar to the AVP group (21.5 [21.0–23.0]) (overall log-rank chi-square = 15.53; $p < 0.005$) (**Fig. 5*A***).

Early Intervention (Comparing the Effects of Selepressin, AVP, and NE Early in the Development of Shock)

Systemic Hemodynamics. The mean time to develop a 10% decrease in MAP was similar in all groups (2.5 ± 0.3 hr, 2.6 ± 0.2 hr, 2.6 ± 0.1 hr, and 2.8 ± 0.7 hr in the selepressin, AVP, NE, and control groups, respectively; p=0.627). Selepressin blunted the increase in heart rate and notably delayed the decrease in cardiac index compared with the control group (**Fig. 2*B***; and **Table S3**, Supplemental Digital Content 1, http://links.lww.com/CCM/B479). MAP was better maintained with selepressin than with AVP or NE, and SVRI and LVSWI were maintained better with selepressin than with NE (Fig. 2*B*; and Table S3, Supplemental Digital Content 1, http://links.lww.com/CCM/B479). The time to develop refractory hypotension in the selepressin group was significantly prolonged compared with the other groups (Table 1), despite a lower fluid balance.

Organ Function and Regional Perfusion. Selepressin delayed the decreases in $Pa_{O_2}/F_{I_{O_2}}$ ratio, lung compliance, and pH (Table S3, Supplemental Digital Content 1, http://links.lww.com/CCM/B479). The lung wet/dry ratio was lower in the selepressin group than in the other groups (**Fig. 3*B***), as was the EVLWI (Table S3, Supplemental Digital Content 1, http://links.lww.com/CCM/B479). The selepressin group also had a less positive cumulative fluid balance (**Fig. S1*B***, Supplemental Digital Content 1, http://links.lww.com/CCM/B479). Less edema formation was also suggested indirectly by the higher colloid oncotic pressure in the selepressin group than in the other groups (**Table S4**, Supplemental Digital Content 1, http://links.

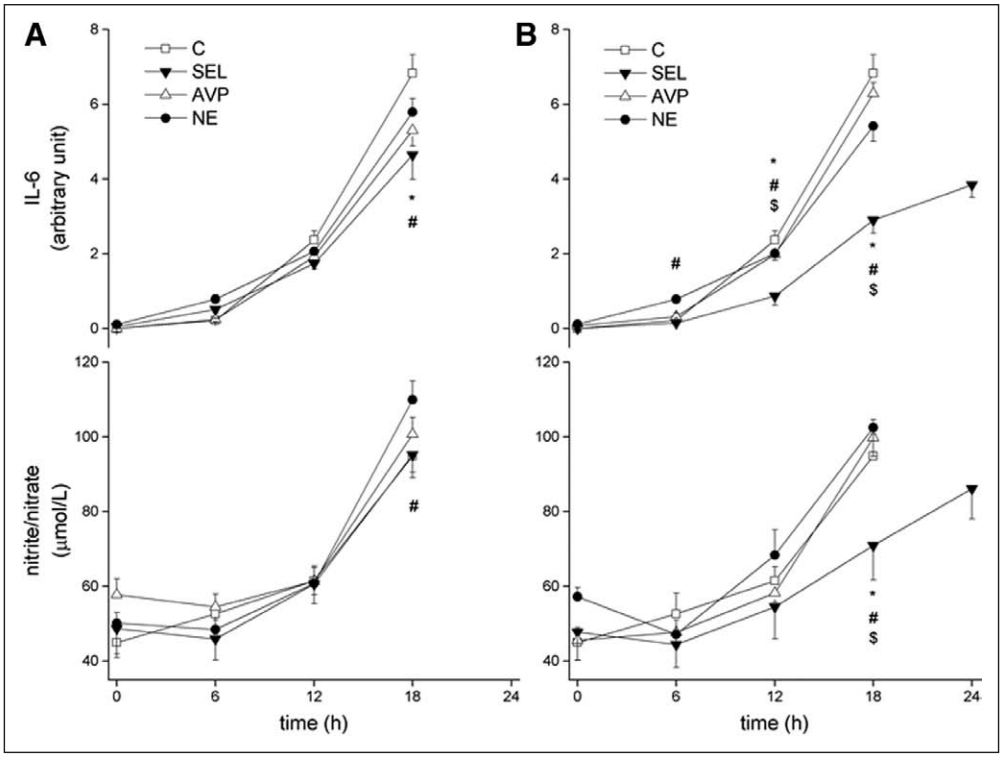

Figure 4. Evolution of plasma interleukin (IL)-6 and nitrite/nitrate concentration in late-intervention group (**A**) and early-intervention group (**B**). Time effects and group × time interaction effects were significant. Group effects with the late intervention were nonsignificant, but, with the early intervention, they were significant for IL-6 but not for nitrite/nitrate. */$/#$p < 0.05$ control group (C), arginine vasopressin (AVP), or norepinephrine (NE) compared with selepressin (SEL), respectively

Renal blood flow and creatinine clearance were higher in the selepressin group than in the NE and control groups (Table S3, Supplemental Digital Content 1, http://links.lww.com/CCM/B479). Oliguria occurred later in the selepressin group than in the other groups (**Fig. S2*B***, Supplemental Digital Content 1, http://links.lww.com/CCM/B479). Changes in mesenteric blood flow were similar among groups (Table S3, Supplemental Digital Content 1, http://links.lww.com/CCM/B479).

Biological Mediators and Plasma Selepressin Concentration. The increases in plasma IL-6 and nitrite/nitrate concentrations were significantly less in the selepressin group than in the other groups (Fig. 3*B*). Plasma selepressin concentrations were 0.19 nmol/L (0.19–0.39), 0.38 nmol/L (0.19–0.40), 0.67 nmol/L (0.40–2.67), and 2.24 nmol/L (1.41–9.03) at 6, 12, 18, and 24 hours, respectively.

lww.com/CCM/B479). Selepressin-treated animals had higher Do_2I and Svo_2 than the NE-treated animals and control animals (Table S3, Supplemental Digital Content 1, http://links.lww.com/CCM/B479). Blood lactate concentrations were also lower in the selepressin-treated animal group than in animals in the other groups (Fig. 2*B*).

Coagulation. Selepressin blunted the increases in PT and aPTT compared with the AVP and control groups. The effects on the evolution of these parameters were greater in the

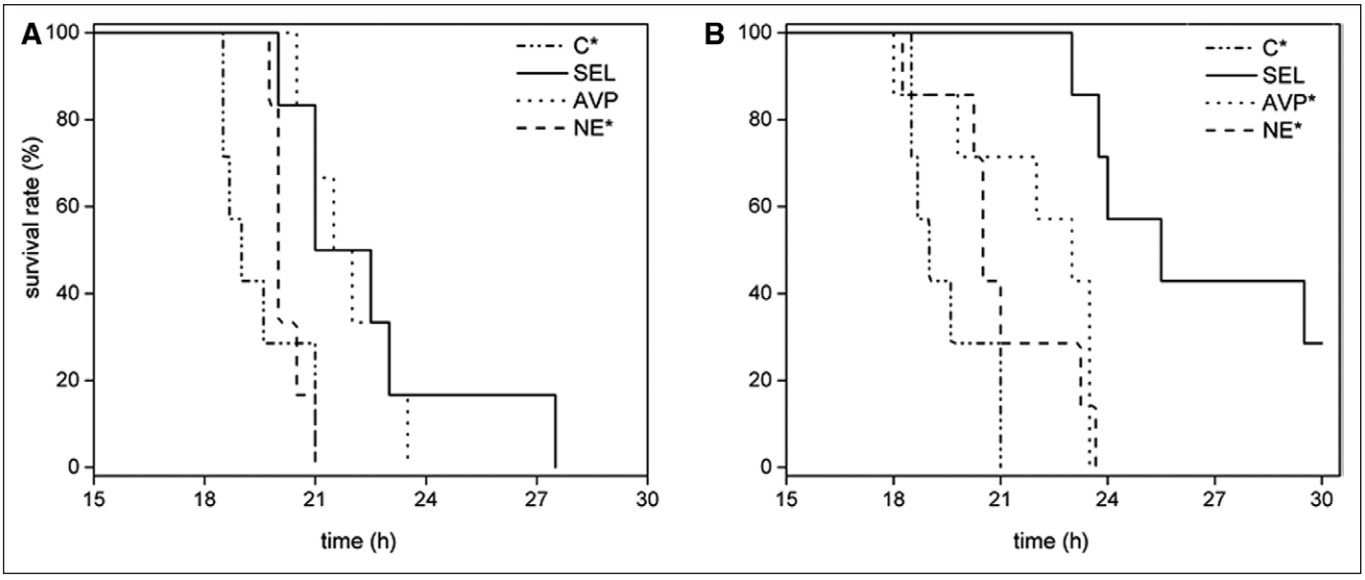

Figure 5. Kaplan-Meier survival curves in late-intervention group (**A**) and early-intervention group (**B**). *$p < 0.05$ compared with selepressin (SEL) group. C = control group, AVP = arginine vasopressin, NE = norepinephrine.

selepressin group (Table S4, Supplemental Digital Content 1, http://links.lww.com/CCM/B479).

Outcome. The median survival time was significantly longer in the selepressin group (25.5 hr [23.8–25.5]) than in the other groups (AVP, 23.0 hr [19.8–23.5]; NE, 20.5 hr [20.3–23.3]; control, 19.0 hr [18.5–21.0]) (log-rank chi-square = 22.75, $p < 0.01$) (**Fig. 5*B***).

DISCUSSION

The main findings of the present study are that 1) administered when septic shock was already established, the selective V_{1A} receptor agonist, selepressin, maintained MAP to a similar extent as did NE and the mixed V_{1A}/V_2 receptor agonist, AVP, but was associated with reduced lung edema compared with these two vasopressor agents and with prolonged survival compared with NE; 2) administered early (i.e., when MAP had decreased by just 10% from baseline), the effects of selepressin were more pronounced, with better hemodynamic stabilization, preserved lung and renal function, reduced cumulative fluid balance, attenuated coagulation disorders, and prolonged survival. In the present model, selepressin was, therefore, superior to AVP or NE in terms of hemodynamic resuscitation, kidney perfusion, and survival; this was associated with reduced systemic inflammation and vascular/capillary leakage.

AVP and its analogs have been shown to exert strong vasopressor effects in several experimental and clinical studies of septic shock (5–7, 23–25). Unlike NE, which increases arterial tone by stimulating vascular α-adrenergic receptors on the peripheral arterioles, AVP and its analogs induce vasoconstriction by stimulating the vascular V_{1A} receptors (9). Although when compared with AVP and NE alone, the AVP analog, terlipressin, a prodrug of lysine vasopressin, also a mixed V_{1A}/V_2 receptor agonist like AVP (26), was associated with better maintained MAP in porcine and ovine models of fecal peritonitis-induced septic shock (5, 27) and in an ovine model of IV endotoxin-induced septic shock (28), as well as in patients with septic shock (25), other studies have suggested that selective stimulation of V_{1A} receptors may be more beneficial than $V_{1A} + V_2$ receptor costimulation in septic shock. [Phe^2,Orn^8] vasotocin, a selective V_{1A} receptor agonist (11), was superior to AVP when added to NE in an ovine model of fecal peritonitis-induced septic shock (14) or infused alone in an ovine model of methicillin-resistant *Staphylococcus aureus* (MRSA) pneumonia-induced sepsis (15). In the present study, the highly selective V_{1A} receptor agonist, selepressin, a full V_{1A} receptor agonist like AVP but with virtually no V_2 receptor agonist activity (10, 29), had stronger vasopressor effects than AVP or NE. Similar observations were made by Maybauer et al (16) in an ovine model of sepsis induced by *Pseudomonas aeruginosa* pneumonia. Because V_2 receptor stimulation can cause vasodilation by inducing NO production from endothelial cells (30), the higher selectivity of selepressin for the V_{1A} receptor may result in less NO being generated, leading to less formation of nitrite and nitrate, which are oxidative metabolites of NO. This effect was demonstrated by Rehberg et al (15) with the selective V_{1A} receptor agonist, [Phe^2,Orn^8]vasotocin, in an ovine model of MRSA pneumonia-induced sepsis. In the present study, we noted lower plasma nitrite/nitrate concentrations in the selepressin group compared with the other groups.

In our study, the three vasopressors did not reduce cardiac index, stroke volume index, or LVSWI, and the early administration of these vasopressors even resulted in increases in these variables. All pure vasopressors can reduce cardiac output (6, 23, 25). AVP and its V_{1A} receptor agonist analogs can reduce myocardial function due to reduced coronary blood flow (31), and the decrease in stroke volume may be associated with a marked increase in afterload (32); this effect is dose-dependent. In studies in porcine fecal peritonitis-induced septic shock (6), ovine endotoxin-induced septic shock (33) and healthy dogs (34), in which AVP or a V_{1A} receptor agonist was infused at low vasopressor doses, myocardial injury was not detected. In a clinical trial comparing terlipressin and AVP, neither intervention significantly affected stroke volume (25). In addition, early administration of selepressin, as in the present study, might mitigate the negative inotropic effects and myocardial injury associated with sepsis by reducing excessive NO and/or cytokine levels (35). This effect was also demonstrated in an ovine model of MRSA pneumonia-induced sepsis, in which administration of [Phe^2,Orn^8]vasotocin was associated with reduced NO concentrations and increased left ventricular contractility (15).

AVP can decrease proinflammatory cytokine release via V_{1A} receptor stimulation (36). Conversely, down-regulation of V_{1A} receptor expression in different tissues can contribute to increases in proinflammatory cytokine release (37). In this context, selective V_{1A} receptor agonism in our study was associated with attenuation of systemic inflammation, especially when given early. Unfortunately, we could not determine the concentrations of many cytokines because there are no specific monoclonal antibodies available in sheep. The anti-inflammatory effects likely contributed to the maintenance of cardiopulmonary function, the limitation of edema formation, and possibly the mitigation of mitochondrial injury (38). As a result, tissue oxygen metabolism was improved, as reflected by lower blood lactate concentrations. The attenuation of coagulopathy by selepressin was also remarkable because selective V_{1A} receptor agonism not only limits the expression of tissue factor induced by IL-6 (39) but also does not induce V_2-receptor-related procoagulant effects mediated by von Willebrand factor release and reduction in fibrinogen levels (13, 29).

Our model is characterized by marked early vascular/capillary leakage, as reflected by a very positive fluid balance and a 50% decrease in total plasma protein concentration already by 2 hours after sepsis induction. A positive fluid balance has been shown to be an independent risk factor for mortality in patients with septic shock (40). In ovine models of sepsis and septic shock induced by fecal peritonitis (14) or MRSA pneumonia (15), administration of the selective V_{1A} receptor agonist, [Phe^2,Orn^8]vasotocin, resulted in reduced vascular leakage and hence less lung edema and better gas exchange than did

AVP or NE. Likewise, in the present study, selepressin-treated animals had less lung edema, as reflected by the lower EVLW and postmortem lung wet/dry weight ratio, compared with the AVP, NE, or control groups, particularly when the drug was administered early.

Renal function was better preserved by selepressin, particularly when administered early. In experimental and clinical studies of septic shock, AVP, terlipressin, or [Phe^2,Orn^8] vasotocin was shown to increase urine output and creatinine clearance (14, 41, 42). Because selepressin cannot induce V_2 receptor-mediated antidiuresis, due to its very high selectivity for the V_{1A} receptor (8, 10), restoration of arterial pressure may have contributed to the preserved renal perfusion and the increased urine output. Improved glomerular filtration, as indicated by better preserved creatinine clearance, could also be related to inhibition of NO generation by selepressin because excessive NO may injure the glomerular endothelium and its metabolism (43). The benefit of the reduction in NO generation on renal function was also observed in ovine sepsis models using [Phe^2,Orn^8]vasotocin (14, 15). Improved renal function may potentially contribute to a less positive fluid balance.

The beneficial effects of AVP analogs on outcome are not definitely proven (4), although a meta-analysis of nine randomized controlled trials (44) suggested that AVP and terlipressin may reduce mortality when compared with NE in patients with septic shock. In our study, early administration of selepressin was associated with much longer survival than early administration of AVP. Early administration of [Phe^2,Orn^8] vasotocin also prolonged survival in an ovine model of peritonitis-induced septic shock similar to that used in the present study (14).

Our study extends the previous observations by Rehberg et al (14, 15) and Maybauer et al (16) and further explores the best timing of administration in the course of sepsis. The effects of selepressin were clearly superior when administration was started early. The advantage of earlier onset of administration has also been suggested in previous studies. A first-line treatment regimen of AVP or terlipressin had better effects than NE alone in ovine (5) and porcine (6) models of fecal peritonitis-induced septic shock and in a predefined subgroup of patients with mild septic shock in the Vasopressin and Septic Shock Trial (4).

The superiority of AVP and V_{1A} receptor agonist analogs over NE may be partly associated with the adverse effects of NE, including stimulation of cellular metabolism, arrhythmias, and myocardial injury. AVP and V_{1A} receptor agonist analogs can also induce vasoconstriction with risks of reduced organ blood flow (34, 45–47). In the present study, we did not observe such adverse effects. Three factors may have contributed to this finding: first, we used generous fluid resuscitation to prevent any fluid deficit; second, the upper limit of the dose ranges of selepressin, AVP, and NE was relatively low in terms of vasopressor action; and third, the animals were previously healthy, so the present observations may not fully apply to patients with comorbidities, including coronary or vascular disease. In addition, antibiotics were not administered in the present study to avoid a confounding factor that may have improved survival.

This study could not determine the precise mechanisms underlying the potential advantages of selepressin, and future studies should focus on this aspect, including measurements of AVP levels, additional biomarkers, and histologic and immunohistochemical examination for evaluation of organ injury, coagulopathy, and vascular barrier dysfunction. Furthermore, as in the clinical setting, some sheep may be highly responsive and others less responsive to vasopressors, and it may be interesting to explore this possibility in future studies.

CONCLUSION

In this clinically relevant model of fecal peritonitis-induced septic shock, the selective V_{1A} receptor agonist, selepressin, was superior to AVP and to NE in the treatment of septic shock. In particular, early administration of selepressin improved the systemic and pulmonary circulations and pulmonary and renal function, attenuated the sepsis-associated coagulation disorder and systemic inflammatory response, and prolonged survival. These observations support the early administration of selective V_{1A} agonists in septic shock as first-line vasopressors, an approach that warrants investigation in clinical trials.

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