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ORIGINAL ARTICLE

Resuscitation with centhaquin and 6% hydroxyethyl starch 130/0.4 improves survival in a swine model of hemorrhagic shock: a randomized experimental study

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Abstract

Purpose To investigate the effects of the combination of centhaquin and 6% hydroxyethyl starch 130/0.4 (HES 130/0.4) in a swine model of hemorrhagic shock.

Methods Twenty Landrace–Large White pigs were instrumented and subjected to hemorrhagic shock. The animals were randomly allocated in two experimental groups, the control (group CO, $n=10$) and the centhaquin groups (0.015 mg/kg, $n=10$, group CH). Acute hemorrhage was induced by stepwise blood withdrawal (18 mL/min) from the internal jugular vein until MAP decreased to 40–45 mmHg, whereas anesthesia remained constant. All animals received HES 130/0.4 solution in the resuscitation phase until their mean arterial pressure (MAP) reached 90% of the baseline. The animals were observed for 60 min, during which no further resuscitation was attempted.

Results The total amount of blood and the bleeding time did not differ significantly between group CO and group CH (120 ± 13 vs. 120 ± 14 mL, $p=0.6$; 20 ± 2 vs. 20 ± 1 min, $p=0.62$, respectively). During the hemorrhagic phase, only a difference in heart rate (97.6 ± 4.4 vs. 128.4 ± 3.6 beats/min, $p=0.038$) was observed between the two groups. The time required to reach the target MAP was significantly shorter in the centhaquin group compared to controls (13.7 ± 0.4 vs. 19.6 ± 0.84 min, $p=0.012$). During the resuscitation phase, a statistical significant difference was observed in MAP (75.2 ± 1.6 vs. 89.8 ± 2.1 mmHg, $p=0.02$) between group CO and group CH. During the observation phase, a statistical significant difference was observed in SVR (1109 ± 32.65 vs. 774.6 ± 21.82 dyn s/cm^5, $p=0.039$) and cardiac output (5.82 ± 0.31 vs. 6.9 ± 0.78 L/min, $p=0.027$) between the two groups. Two animals of group CO and seven animals of group CH survived for 24 h ($p=0.008$). We observed a marked increase in microvascular capillary permeability in group CO compared to group CH, with the wet/dry weight ratio being significantly higher in group CO compared to group CH (4.8 ± 1.6 vs. 3.08 ± 0.6, $p<0.001$).

Conclusions The combination of centhaquin 0.015 mg/kg and HES 130/0.4 resulted in shorter time to target MAP, lower wet-to-dry ratio, and better survival rates after resuscitation from hemorrhagic shock.

Keywords Hemorrhagic shock · Centhaquin · Hydroxyethyl starch · Acute care anesthesiology · Emergency surgery · Survival

Introduction

Millions of people die annually due to major trauma and hemorrhagic shock. In these patients, marked depletion of intravascular volume leads to severe hemodynamic instability, decreased cardiac output, impaired oxygen delivery, and organ failure [1]. Treatment strategies include adequate fluid resuscitation, transfusion of compatible blood, and surgical repair of the reversible causes of hemorrhage [1–3].

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Nevertheless, volume loading varies between trauma centers due to the scarcity of published evidence and lack of randomized controlled clinical trials.

The amount of crystalloids needed to increase mean arterial pressure (MAP) is usually large and often associated with various complications, such as acute lung injury (ALI), pulmonary edema, and multiple organ failure [4]. This led the scientific community to focus on resuscitation with smaller volumes and colloids [5–8], and hydroxyethyl starch solutions have evolved to become the most commonly used colloid [9]. These solutions have been popular, because they are more efficient volume expanders than crystalloids and may achieve better microcirculatory recruitment than normal saline [10, 11].

Centhaquin citrate is a novel agent that augments cardiac output and reduces systemic vascular resistance (SVR), which may be crucial for the preservation of tissue perfusion [12–15]. We have recently shown that centhaquin 0.015 mg/kg administered together with lactated Ringer's (R-L) solution during resuscitation from hemorrhagic shock resulted in lower volume of fluids and better survival compared to control and sham-operated animals [15]. On the other hand, colloids have been considered to be four to fivefold more effective than crystalloids in expanding blood volume [16]. As colloids are related to a lower fluid balance than crystalloids, the co-administration of centhaquin and colloids seems intriguing. The purpose of this study was to investigate the effects of the combination of centhaquin and 6% hydroxyethyl starch 130/0.4 (HES 130/0.4) in a swine model of hemorrhagic shock.

Materials and methods

The protocol was approved by the Hellenic Veterinary Services (license no. 7157/30-11-2012). Twenty pigs of conventional microbiologic status with an average weight 20 ± 1 kg (aged 10–12 weeks) were the study subjects. All animals received anesthetic and surgical procedures in compliance with the Guide for the Care and Use of Laboratory Animals.

All pigs were purchased from the same breeder (Validakis, Koropi, Greece) and were transported 1 week before experimentation to the research facility (Experimental-Research Center ELPEN, European Ref. Number EL 09 BIO 03). The animals were fasted the day before the experimentation, but they had free access to water. Centhaquin citrate (Lot # PMZ-2010/2012/09A) was synthesized at Pharmazz India Private Limited, Greater Noida, India (courtesy Dr. Manish Lavhale).

The experimental protocol has been previously described [15, 17]. In brief, the animals were premedicated with intramuscular ketamine hydrochloride (Merial, Lyon, France) 10 mg/kg, midazolam (Roche, Athens, Greece) 0.5 mg/kg, and atropine sulphate (Demo, Athens, Greece) 0.05 mg/kg and were subsequently transported to the operation research facility. Intravascular access was obtained through the auricular veins and induction of anesthesia was achieved with an intravenous bolus dose of propofol (Diprivan 1% w/v; AstraZeneca, Luton, UK) (2 mg/kg) and fentanyl (Janssen Pharmaceutica, Beerse, Belgium) (2 μg/kg). While breathing spontaneously, the animals were intubated (always by the same researcher) with a size 6.0-mm cuffed endotracheal tube. The endotracheal tube was secured on the lower jaw, and successful intubation was ascertained by auscultation of both lungs, while ventilated with a self-inflating bag.

The animals were then immobilized in the supine position on the operating table. Additional propofol 1 mg/kg, cisatracurium (Nimbex 2 mg/mL GlaxoSmithKline, Athens, Greece) 0.15 mg/kg, and fentanyl 0.01 mg/kg were administered to ascertain synchrony with the ventilator. The animals were mechanically ventilated (Siare Alpha–Delta Lung Ventilator; Siare s.r.l. Hospital Supplies, Bologna, Italy—Volume controlled ventilation, tidal volume 7 mL/kg, I:E 1:2, PEEP 0 cmH_20) with a gas mixture of 40% oxygen and anesthesia was maintained with infusion of propofol 150 μg/kg/min. [15] Normocapnia was achieved using continuous monitoring of end-tidal CO_2 (Tonocap TC-200-22-01; Engstrom Division, Instrumentarium Corp, Helsinki, Finland), and the respiratory rate was adjusted to maintain end-tidal CO_2 ($ETCO_2$) 35–40 mmHg. Pulse oximetry (SpO_2) was monitored throughout the experiment. Body temperature was monitored by a rectal temperature probe and was maintained between 38.5 and 39.5 °C with a heating blanket.

Electrocardiographic monitoring was used, using leads I, II, III, aVR, aVL, and aVF, which were connected to a monitor (Mennen Medical, Envoy; Papapostolou, Athens, Greece). The monitor electronically calculated the heart rate. For measurement of the aortic pressure, an arterial catheter (model 6523, USCI CR, Bart; Papapostolou) was inserted and forwarded into the descending aorta after surgical preparation of the right internal carotid artery. The systolic (SAP) and diastolic (DAP) arterial pressures were recorded, whereas MAP was determined by the electronic integration of the aortic blood pressure waveform. The right internal jugular vein was cannulated with a catheter to measure central venous pressure (CVP). The left internal jugular vein was also surgically prepared, and a catheter was inserted for fluid administration. Intravascular catheters were attached to pressure transducers that were aligned to the level of the right atrium and were calibrated before their use. This allowed the recording of CVP and arterial pressures. Cardiac output was measured as the product of time–velocity integral of Doppler transaortic flow, the diameter of the aortic valve, and heart rate, as previously described [18], while SVR was calculated using the formula SVR = (MAP − CVP)/CO × 80, as previously described [14]. Arterial blood gases

were measured on a blood–gas analyzer (IRMA SL Blood Analysis System, Part 436301; Diametrics Medical Inc, Roseville, MN 55113, pH, pO_2, pCO_2).

The protocol simulated a civilian trauma scenario and has been previously described; it was divided into five distinct phases: stabilization, hemorrhagic, maintenance, resuscitation, and observation phase [19]. The stabilization phase lasted for approximately 30 min after the instrumentation of the animals. Acute hemorrhage was induced by stepwise blood withdrawal (18 mL/min) from the internal jugular vein until MAP decreased to 40–45 mmHg, whereas anesthesia remained constant. During the maintenance phase (60 min), no resuscitation was attempted and the animals were randomly allocated to two experimental groups of ten animals each, the control (group CO) and the centhaquin plus colloid group (group CH). During the resuscitation phase, the animals of group CO received HES 130/0.4 solution, while the animals of group CH received HES 130/0.4 plus centhaquin (0.015 mg/kg). In both groups, fluid infusion was continued until their MAP reached 90% of the baseline. The total amount of fluids and the time required to achieve the target MAP were recorded. The observation phase lasted for 60 min, during which no further resuscitation was attempted.

After data collection, all catheters were removed, the ventilator was switched to manual mode, and the animals were ventilated with a self-inflating bag and mask with a reservoir bag. When the first spontaneous swallowing reflex was detected, neostigmine (0.04 mg/kg) and atropine (0.01 mg/kg) were administered. After adequate inspiration depth was ascertained and SpO_2 measurement was $>97\%$, the animals were extubated, while the vital signs continued to be monitored throughout recovery. After the appearance of the righting reflex, the animals were returned to their cages.

All surviving animals were neurologically assessed 24 h later with a score previously described [18] and euthanatized with an intravenous bolus dose of propofol 40 mg followed by 2 g of thiopental. Lung tissue samples were taken from dependent and nondependent parts of both lower and upper lobes. They were immersed in 10% paraformaldehyde for at least 72 h, dehydrated with graded alcohol, embedded in paraffin, and cut in a series of 4 μm-thick slices that were stained with hematoxylin and eosin [17]. Histologic analyses of tissue samples were performed in a blinded fashion. Tissue samples were analyzed in a blinded fashion according to a previously described scoring system [20]. Features evaluated were focal thickening of the alveolar membranes, vascular congestion, alveolar edema, interstitial neutrophil infiltration, intra-alveolar neutrophil infiltration, and alveolar hemorrhage. To eliminate observer bias, the samples were evaluated simultaneously by two independent pathologists, blinded to the allocation of each animal. To assess the lung wet/dry ratio, which represents the percentage of tissue water and is an index of tissue microvascular permeability, lung samples were also collected after the swine were euthanized. Excess fluid was blotted from the samples, and wet weights were measured in the operating room. Dry weights were measured after drying samples at 80 °C for 72 h.

Pulmonary capillary leakage was determined with the Evans blue dye extravasation method. Specifically, 2% Evans blue dye (20 mg/kg; Sigma Chemical, St. Louis, MO) was injected via a peripheral vein 15 min before swine euthanasia. The lung tissues were excised and weighed. Each sample of tissues was incubated at 37 °C for 24 h after 4.0 mL formamide was added. If necessary, the incubation time was prolonged until the blue color of the samples completely disappeared. After filtration with a glass filter, the absorbance of the filtrate was measured at 620 nm in a Beckman spectrophotometer (Beckman Coulter, Inc., Brea, CA). The total amount of dye can be calculated by means of a standard calibration curve. Microvascular permeability in the lungs was shown as the micrograms (μg) of Evans blue dye in every milligram (mg) of tissue. The pathologists who assessed microvascular permeability were also blinded to the allocation of each animal.

Statistical analysis

Statistical analysis was processed using SPSS Statistics 18.0 for MAC. Continuous variables are expressed as mean ± standard error (mean ± SEM). Scatter plots using means and SEM were produced for all variables and phases of interest. Kaplan–Meier plots are used for presenting survival data, while log-rank tests (Mantel–Cox test) are applied for comparing survival times between centhaquin and control group. One-way ANOVA was used for comparing variables for stages 1–3, while repeated measures ANOVA applied for comparing the differences of variables and the effect of the drug for stages 4, 5 and combined 4 and 5 together. Groups were compared in phase 1 and p values for every parameter was calculated (using T independent test) to have same starting points. A number of ten swine per arm (control and centhaquin groups) were calculated to be sufficient using the two independent proportions sample size estimation. We used all data from all animals for the hemodynamics and we have not excluded any animals from the statistical analysis during the resuscitation period. Parameters of estimation were: the two tails approach, a difference in mortality of 20% between centhaquin vs. control group 80%, power 80% and $\alpha = 0.05$. A level of $p = 0.05$ was set as a level of statistical difference acceptance.

Results

During the stabilization phase, there was a statistically significant difference in DAP and heart rate between the two groups (65 ± 2.3 vs. 88.9 ± 1.9 mmHg, $p = 0.002$; 95 ± 6.6 vs.

114 ± 9.2 beats/min, $p = 0.002$, respectively). At the end of this period, CVP was not different between the two groups (Fig. 1).

The total amount of blood and the bleeding time did not differ significantly between group CO and group CH (120 ± 13 vs. 120 ± 14 mL, $p = 0.6$; 20 ± 2 vs. 20 ± 1 min, $p = 0.62$, respectively). During the hemorrhagic phase, only a difference in heart rate (97.6 ± 4.4 vs. 128.4 ± 3.6 beats/min, $p = 0.038$) was observed between the two groups. Although the total amount of the infused HES 130/0.4 was not significantly different between the two groups (group CO: 512 ± 37 mL, group CH: 389 ± 41 mL; $p = 0.204$), the time required to reach the target MAP was significantly shorter in the centhaquin group compared to controls (13.7 ± 0.4 vs. 19.6 ± 0.84 min, $p = 0.012$) (Fig. 2). In addition, a statistical significant difference was observed in SAP (96.2 ± 3.6 vs. 108.8 ± 2.5 mmHg, $p = 0.01$), DAP (65.8 ± 2.1 vs. 77.4 ± 1.9 mmHg, $p = 0.045$), and MAP (75.2 ± 1.6 vs. 89.8 ± 2.1 mmHg, $p = 0.02$) (Fig. 2) in this phase (Table 1).

During the observation phase, a statistical significant difference was observed in SVR (1109 ± 32.65 vs. 774.6 ± 21.82 dyn s/cm^5, $p = 0.039$) (Fig. 3) and cardiac output (5.82 ± 0.31 vs. 6.9 ± 0.78 L/min, $p = 0.027$) (Fig. 4) between the two groups. At the end of the observation phase, the Horowitz index was 327 ± 10 and 392 ± 16 in the group CO and CH, respectively. Two animals of group CO and seven animals of group CH survived for 24 h ($p = 0.008$). Specifically, in group CO, two animals died after 6 h, one after 8 h, three after 11 h, one after 14 h, and one after 15 h from the onset of hemorrhagic shock. In group CH, one animal died after 11 h, one after 14 h, and one after 16 h from the onset of shock. We observed a marked increase in microvascular capillary permeability in group CO compared to group CH, with the wet/dry weight ratio being significantly higher in group CO compared to group CH (4.8 ± 1.6 vs. 3.08 ± 0.6, $p < 0.001$).

Discussion

Although fluid administration is one of the most important parts of hemorrhagic shock resuscitation and has been the standard of care for years, it remains a controversial issue due to the related adverse effects [21]. Liberal crystalloid administration may affect base deficit and worsen coagulopathy, hypothermia, and acidosis [22–24], while colloids have been reported to increase the risk for death compared to crystalloids, especially in large amounts [25, 26].

Recent evidence suggests that the modern HES 130/0.4 may not be as detrimental as it was currently believed [27–29], but the debate on this issue remains inconclusive. In this context, minimizing the administered amount of HES 130/0.4 may allow to exploit its advantages while decreasing the possibility of complications [30, 31]. In the present study, we investigated the effects of the combination of centhaquin and HES 130/0.4 in a swine model of hemorrhagic shock and observed that the animals receiving centhaquin required fewer fluids, restored their arterial pressures quicker, and exhibited improved survival compared with animals receiving HES 130/0.4 alone.

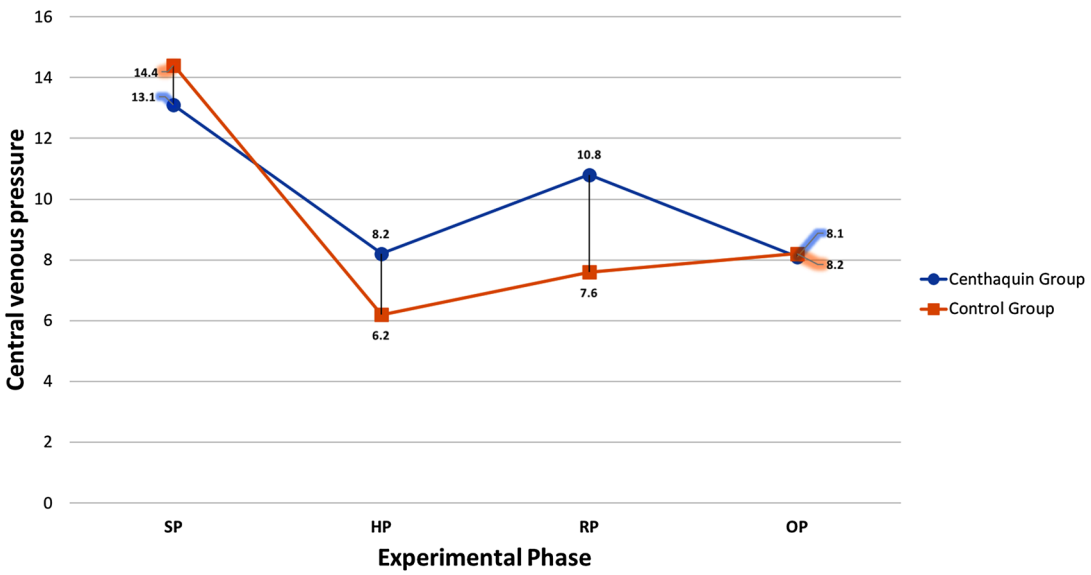

Fig. 1 Variations of central venous pressure during the experiment. *SP* stabilization phase, *HP* hemorrhagic phase, *RP* resuscitation phase, *OP* observation phase

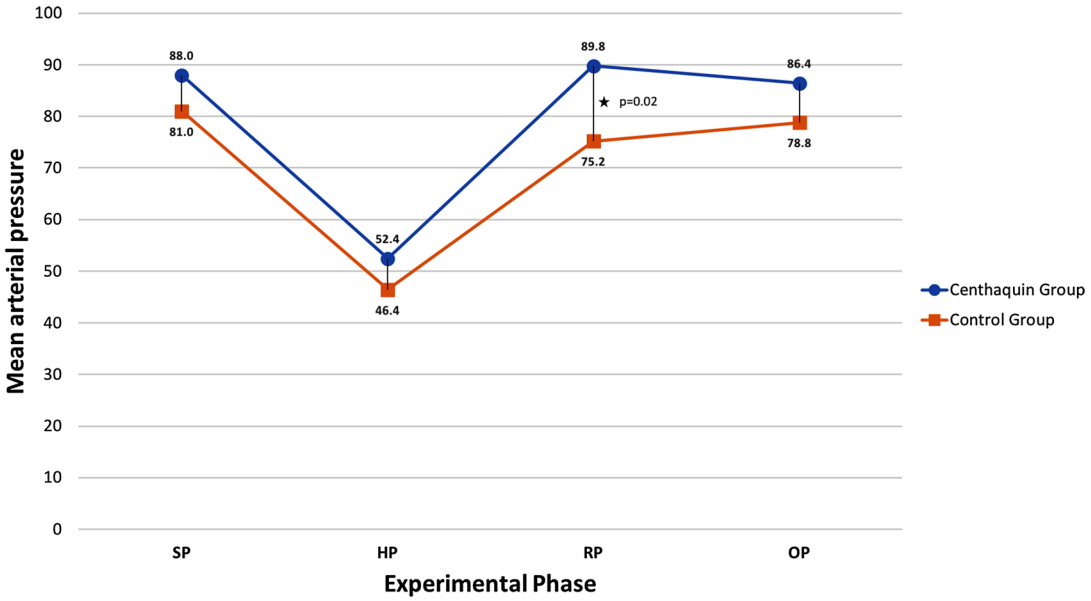

Fig. 2 Differences in mean arterial pressure between the two groups during the experiment. *SP* stabilization phase, *HP* hemorrhagic phase, *RP* resuscitation phase, *OP* observation phase

Although the a-adrenergic effects of centhaquin may result in shorter time to target MAP [15, 32–35], we did not observe a statistically significant difference in SVR between the two groups during the resuscitation phase. Considering the smaller amount of fluids in group CH, significant increases in cardiac inotropy and chronotropy would be required to justify the statistically significant increase in MAP in this group compared to group CO.

During hypovolemia, the hydrostatic capillary pressure is lower than normovolemia, tending to reduce fluid shifting towards the interstitial space [30]. Although the high molecular weight of HES 130/0.4 increases the plasma oncotic pressure [36], it may offer no hemodynamic advantages compared to R-L in hypovolemic individuals with low capillary pressure [15, 31]. Therefore, another mechanism may be responsible for the insignificant difference in SVR between group CO and group CH during the resuscitation phase. The most possible reason for this finding may be the effect of centhaquin on the unstressed volume of the group CH animals. The a-adrenergic properties of centhaquin may recruit volume form the unstressed compartment, increasing the stressed volume and improving hemodynamics without large increases in SVR [37–39]. In addition, centhaquin has central sympatholytic activity, which reduces the hemorrhage-induced vasoconstriction and SVR and preserves tissue perfusion [12, 15]. The latter is also enhanced by the recruitment-induced decrease in splachnic venular pressure, which enhances capillary blood flow and tissue oxygenation [13–15].

Previous studies demonstrated that different types of fluids affect the inflammatory response and are involved in the pathogenesis of multiple organ failure [40]. A major cause of hemorrhage-induced interstitial edema and acute lung injury (ALI) is fluid overload, with the main mechanism being the ischemia/reperfusion-induced inflammatory response and oxidative stress [41]. In this study, we observed a marked increase in microvascular capillary permeability in group CO compared to group CH, with the wet/dry weight ratio being significantly higher in group CO (4.8 ± 1.6 vs. 3.08 ± 0.6, $p < 0.001$). In addition, microvascular permeability and wet/dry weight ratio in group CH were significantly lower than these in our previous experiments using either lactated R-L or HES 130/0.4 alone [15, 19]. In the literature, several studies have reported that the use of HES may reduce the pulmonary inflammatory response, oxidative stress, and the interstitial edema caused by increased capillary permeability [40, 42–47]. Our results show for the first time that these effects may be enhanced by the co-administration of centhaquin in prolonged hemorrhagic shock [48].

Our study has several limitations. First, our model includes healthy animals and does not consider the associated traumatic injuries. Second, the hemodynamic response to hemorrhage may have been affected by the general anesthesia; the effects of centhaquin may be more prominent in

Table 1 Hemodynamic parameters of the animals

Variable	Stabilization phase			Hemorrhagic phase			Resuscitation phase			Observation phase		
	Group CO	Group CH	*p* value	Group CO	Group CH	p value	Group CO	Group CH	*p* value	Group CO	Group CH	*p* value
SAP (mmHg)	107.6 ± 4.5	115.4 ± 3.2	0.119	62.8 ± 2.3	72.1 ± 2.1	0.071	96.2 ± 3.6	108.8 ± 2.5	0.01	103.6 ± 4.6	99.8 ± 3.2	0.574
DAP (mmHg)	65 ± 2.3	88.9 ± 1.9	0.002	36.6 ± 2.4	45.8 ± 2.3	0.170	65.8 ± 2.1	77.4 ± 1.9	0.045	65.4 ± 3.7	70 ± 3.1	0.373
MAP (mmHg)	81 ± 4.6	88 ± 5.9	0.135	46.4 ± 1.7	52.4 ± 1.5	0.356	75.2 ± 1.6	89.8 ± 2.1	0.02	86.4 ± 1.8	78.8 ± 2.2	0.265
CVP (mmHg)	14.4 ± 0.8	13.1 ± 1.1	0.288	6.2 ± 0.9	8.2 ± 0.8	0.161	7.6 ± 0.7	10.8 ± 0.4	0.251	8.2 ± 0.5	8.1 ± 0.6	0.981
HR (beats/min)	95 ± 6.6	114 ± 9.2	0.002	97.6 ± 4.4	128.4 ± 3.6	0.038	127.2 ± 3.1	122.3 ± 3.8	0.471	126.8 ± 2.4	126.3 ± 1.9	0.948
SVR (dyn s/cm^5)	1096 ± 76.16	1240 ± 93.12	0.522	908 ± 34.52	927.3 ± 29.3	0.930	1082 ± 28.63	907.3 ± 32.6	0.355	1109 ± 32.65	774.6 ± 21.82	0.039
Cardiac output (L/min)	4.88 ± 0.2	5.05 ± 0.14	0.643	3.46 ± 0.12	4.2 ± 0.42	0.118	5.44 ± 0.11	7.16 ± 0.23	0.074	5.82 ± 0.31	6.9 ± 0.78	0.027

Data are presented as mean ± SD

SAP systolic arterial pressure, *DAP* diastolic arterial pressure, *MAP* mean arterial pressure, *CVP* central venous pressure, *HR* heart rate, *SVR* systemic vascular resistance

the absence of anesthetic agents. Another limitation is that no sham group was studied. However, the General Directorate of Veterinary Services would not grant us approval for such a group, as the expected mortality of the animals during the hemorrhagic phase would be extremely high. Thus, following the universally accepted 3 Rs (reduction, refinement, replacement) in experimental research, the smallest number of animals was sought to test our hypothesis. Nevertheless, in our previous study, we added a sham group and confirmed the effectiveness of centhaquin regardless of the duration of shock [15]. Furthermore, the sample of the present study achieves a power of 80%. Another limitation is that we were not able to measure any inflammatory markers, important physiological data on tissue perfusion, and the long-term effects after this treatment, making it particularly difficult to draw any safe conclusions regarding the mechanism of the long-term beneficial effects of centhaquin. Finally, we used one dose of centhaquin, and therefore, we are unable to comment whether different doses may have yielded different effects in this swine model of controlled hemorrhage.

Conclusions

The combination of centhaquin 0.015 mg/kg and HES 130/0.4 resulted in shorter time to target MAP, lower wet-to-dry ratio, and better survival rates after resuscitation from hemorrhagic shock.

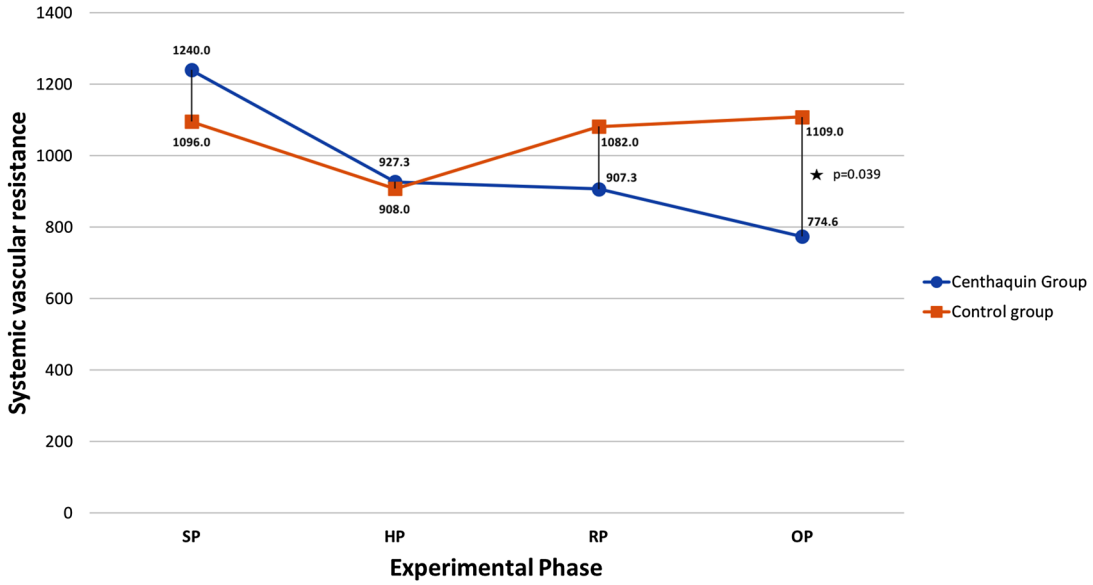

Fig. 3 Changes in systemic vascular resistance during the experiment. *SP* stabilization phase, *HP* hemorrhagic phase, *RP* resuscitation phase, *OP* observation phase

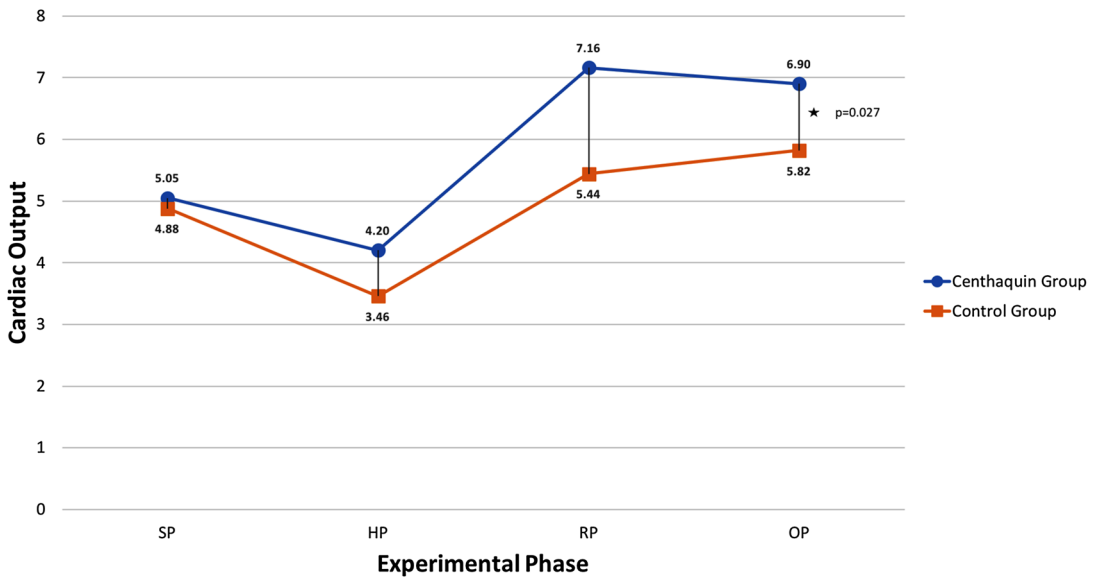

Fig. 4 Differences in cardiac output between the two groups during the experiment. *SP* stabilization phase, *HP* hemorrhagic phase, *RP* resuscitation phase, *OP* observation phase

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Compliance with ethical standards

Conflict of interest Zinais Kontouli, Chryssoula Staikou, Nicoletta Iacovidou, Ioannis Mamais, Evaggelia Kouskouni, Apostolos Papalois, Panagiotis Papapanagiotou, Anil Gulati, Athanasios Chalkias, and Theodoros Xanthos declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Non-applicable. We did not include patients in this study.

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