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Effect of physostigmine on recovery from septic shock following intra-abdominal infection – Results from a randomized, double-blind, placebo-controlled, monocentric pilot trial (Anticholium® per Se)

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ABSTRACT

Purpose: The cholinergic anti-inflammatory pathway has been shown to be accessible by physostigmine salicylate in animal models. However, the cholinesterase inhibitor is not approved for adjunctive therapy in sepsis, and tolerability and safety of high initial doses followed by continuous infusion have not been investigated.
Materials and methods: In this trial, 20 patients with perioperative septic shock due to intra-abdominal infection were eligible. The physostigmine group received an initial dose of 0.04 mg/kg physostigmine salicylate, followed by continuous infusion of 1 mg/h for 120 h; the placebo group was treated with 0.9% sodium chloride. Primary outcome was the mean Sequential Organ Failure Assessment (SOFA) score during treatment and up to 14 days.
Results: Administration of physostigmine salicylate was well tolerated. Mean SOFA scores were 8.9 ± 2.5 and 11.3 ± 3.6 (mean ± SD) for physostigmine and placebo group, respectively. Adjusted for age, difference between means was not statistically significant (−2.37, 95% CI: −5.43 to 0.70, $p = 0.121$). Norepinephrine doses required only appeared lower in the physostigmine group ($p = 0.064$), along with a more rapid reduction from an elevated heart rate possibly indicating less hemodynamic instability.
Conclusions: Treatment with physostigmine salicylate was feasible and safe. Further studies are justified to assess the effect on recovery from septic shock.
Trial registration: EudraCT Number 2012-001650-26, ClinicalTrials.gov identifier NCT03013322.

1. Introduction

Affecting millions of people worldwide, sepsis is a global public health burden and remains a major challenge, despite advances in intensive care [1,2].

Sepsis is a clinical syndrome characterized by multiple pathophysiological abnormalities [3]. The current definition of sepsis (Sepsis-3) focuses on life-threatening organ dysfunction caused by a dysregulated host response to infection; organ dysfunction is defined as an increase of two or more points in the Sequential Organ Failure Assessment (SOFA) score [3-5].

Septic diseases may lead to multiple organ dysfunction and hospital mortality rates are high, reaching approximately 50.7%–59% for septic shock [6,7]. In light of these alarming figures, new treatment options are of global interest, and research to identify novel approaches is

Abbreviations: α7nAChR, Alpha7 nicotinic acetylcholine receptors; ANCOVA, Analysis of covariance; APACHE, Acute Physiology And Chronic Health Evaluation; BfArM, Federal Institute for Drugs and Medical Devices; BMI, Body mass index; bpm, Beats per minute; CTCAE, Common Terminology Criteria for Adverse Events; GCS, Glasgow Coma Scale; ICU, Intensive care unit; IL, Interleukin; IMBI, Institute of Medical Biometry and Informatics; IMP, Investigational medicinal product; IOPIS, Interdisciplinary Operative Intensive Care Unit; IQR, Interquartile range; KKS, Coordination Centre for Clinical Trials; MAP, Mean arterial blood pressure; MedDRA, Medical Dictionary for Regulatory Activities; PT, Preferred Terms; QTc, Corrected QT interval; RRT, Renal replacement therapy; SAPS, Simplified Acute Physiology Score; SIRS, Systemic inflammatory response syndrome; SD, Standard deviation; SOC, System Organ Class; SOFA, Sequential Organ Failure Assessment; TNF, Tumor necrosis factor.

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underway. While some potential therapies remain limited to animal models, others have been transferred from bench to bedside, but failed to prove their benefits in humans [8-10].

The autonomic nervous system has been a focus of interest for many years, introducing the cholinergic anti-inflammatory pathway [11-13]. The concept is based on an inflammatory reflex of the vagus nerve, by which the parasympathetic nervous system may suppress systemic inflammation and thereby protect against cytokine-mediated diseases [14]. Modulation of the immune system through cholinergic mechanisms was found to be mediated by the α-bungarotoxin-sensitive nicotinic alpha7 acetylcholine receptors (α7nAChR) on macrophages and immune cells [11,13,15]. Studies using rat and mouse models have observed that peripheral vagus nerve stimulation or treatment with physostigmine salicylate inhibited inflammatory response, such as release of tumor necrosis factor (TNF)-α and interleukin (IL)-1β; severe hypotension following endotoxemia or cecal ligation and puncture was reduced significantly, preventing progression from sepsis to severe sepsis and septic shock [11,16].

Based on these preclinical observations, this pilot study, Anticholium® per Se, addressed whether treatment with physostigmine salicylate targeting the cholinergic anti-inflammatory pathway could be transferred from bench to bedside, and if this intervention was feasible and safe. Thus, it investigated the effect of treatment with physostigmine salicylate or placebo on the mean SOFA score during treatment and subsequent intensive care of up to 14 days in patients with perioperative septic shock due to intra-abdominal infection.

2. Materials and methods

Trial design and setting; participants; all processes, interventions and comparisons; outcomes; sample size; randomization and blinding; and informed consent are detailed in the protocol published previously [17].

The clinical trial had been registered at the EU Clinical Trials Register (EudraCT Number: 2012-001650-26) and clinicaltrials.gov (NCT03013322). The study was conducted in accordance with the Declaration of Helsinki. Study protocol and corresponding documents had been approved by the Federal Institute for Drugs and Medical Devices (BfArM) and the Ethics Committee, Medical Faculty of Heidelberg University (AFmu-447/2012). Before enrollment, informed consent from a legal guardian, a near family member to be designated legal guardian, or a guardianship judge was obtained [17].

2.1. Trial design

In brief, 20 patients with perioperative septic shock due to intra-abdominal infection were enrolled in this randomized (1:1), double-blind, placebo-controlled, monocentric pilot trial.

2.2. Participants

Adults (aged 18–85 years) with perioperative sepsis (confirmed intra-abdominal infection plus SIRS criteria) and septic shock (<24 h), requiring vasopressor therapy despite adequate fluid resuscitation (according to Sepsis-1 criteria [18]) were eligible. Patients were excluded if they had an Acute Physiology And Chronic Health Evaluation (APACHE) II score ≥ 34 or an unfavorable prognosis of a primary or concomitant illness, expecting the death within the follow-up phase. Further exclusion criteria were contraindications for treatment with physostigmine salicylate (Anticholium®, Dr. Franz Köhler Chemie GmbH, Bensheim, Germany), having undergone splenectomy or solid organ transplantation, and pregnancy or lactation. Detailed eligibility criteria are given in the Supplements.

The study was conducted at the Department of Anesthesiology, Heidelberg University Hospital, Interdisciplinary Operative Intensive Care Unit (IOPIS).

2.3. Interventions

The protocol stipulated enrollment within 24 h of onset of shock, and treatment with the investigational medicinal product (IMP) within the following 2 h. The physostigmine group received an initial dose of 0.04 mg/kg physostigmine salicylate as a short infusion (with a maximum dose of 4 mg), followed by continuous infusion of 1 mg/h (=2.5 mL/h) for 120 h; the placebo group was treated with equivalent volumes of 0.9% sodium chloride in an identical pharmaceutical form and with an identical schedule.

2.4. Outcomes

Feasibility of patient recruitment, administration of IMP, and safety of the intervention were evaluated. A flow chart of trial-specific procedures, assessments, and visits may be found in the Supplements for the reader's convenience.

The mean SOFA score during treatment and subsequent intensive care of up to 14 days was used as surrogate outcome [17,19]. The primary endpoint was calculated as the mean of all daily SOFA scores for each patient, ascertained from visit 2 to 16 (i.e. from 2 h ± 30 min to 14 d ± 8 h after the initial dose of IMP), discharge from the intensive care unit (ICU), or premature study termination including death, whichever occurred first.

Secondary outcome measures included need for supportive therapies (vasopressor support, mechanical ventilation, and renal replacement therapy), 30- and 90-day mortality, and the occurrence of side effects. Laboratory values; including parameters of infection, coagulation, renal and hepatic function; were assessed for minimum and maximum values during the 24 h period prior to the respective visit. Maximum duration of follow-up was limited to visit 20. However, participants left the study prior to 90 days if they were discharged from ICU (completed final visit), transferred to another ICU, or died in ICU (premature termination).

Adverse events were reported as published before [17]. Harms-related information was collected at every visit during and up to 24 h after IMP administration.

2.5. Sample size

This was a pilot study which precludes educated estimates. Planned with a sample size of 10 patients per group, a standardized effect (Cohen's d) of 1.32 at a significance level of $\alpha = 5\%$ with a power of $1 - \beta = 80\%$ was expected to be shown [17].

2.6. Randomization and blinding

According to a computer-generated randomization list in blocks of 2, trial medication prepacked in sequentially numbered containers was provided by Dr. Franz Köhler Chemie GmbH and consecutively allocated to participants [17]. Investigator and members of the study group, healthcare providers, and participants were kept blinded during the clinical phase of the trial [17]. The randomization sequence was not revealed until lock of database. Emergency envelopes at the site remained untouched.

2.7. Statistical analysis

The primary endpoint was the mean SOFA score (at least two individual values) during treatment and subsequent intensive care of up to 14 days. The two-sided analysis of covariance (ANCOVA) model included treatment with physostigmine salicylate as factor and age as continuous covariate [17].

Categorical data were analyzed with the chi-square test. For further continuous/metric data, the Wilcoxon-Mann-Whitney test was used if the respective outcome parameter was available for at least four

patients per group. In addition to the results of the statistical tests, *p*-values and effect estimators with 95% confidence intervals (CI) are given where possible.

Time-to-event and survival data were analyzed using the Kaplan-Meier method and Log-rank test. Time-to-event analysis was performed for the following events: need for supportive therapies (vasopressor support, mechanical ventilation, and renal replacement therapy), cure of sepsis and underlying disease, duration of stay in ICU and hospital, and survival; and comprised time from randomization (i.e. commencement of treatment with IMP) to end of the respective therapy, condition, or death, or end of trial participation due to discharge from ICU or premature study termination. Need for vasopressors was further analyzed for duration of norepinephrine therapy (any dose) defined as time from randomization to end of norepinephrine administration, and duration of septic shock defined as necessary vasopressor therapy (including norepinephrine or epinephrine >0.1 μg/kg/min) and calculated from the time of randomization to the end of the respective vasopressor dose.

The safety analysis population equaled the full analysis population and comprised all randomized participants who had received at least one dose of trial medication. The intention-to-treat population included all patients of the full analysis population from whom the primary outcome measure had been obtained. The per-protocol population consisted of all participants from the intention-to-treat population without serious protocol violations which included violations of eligibility criteria or derivations from the treatment scheme, especially falling short of the minimum duration of administration (48 ± 2 h).

For safety analyses, patients were grouped as treated. Adverse events and medication-related adverse events were reported for each group. Serious adverse events were coded according to the Common Terminology Criteria for Adverse Events (CTCAE) with the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) in System Organ Classes (SOC) and Preferred Terms (PT).

Data analysis was performed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The datasets used or analyzed during the clinical trial are available from the corresponding author on reasonable request.

3. Results

3.1. Baseline data

Between January 2015 and February 2017, 20 patients received the IMP (Fig. 1).

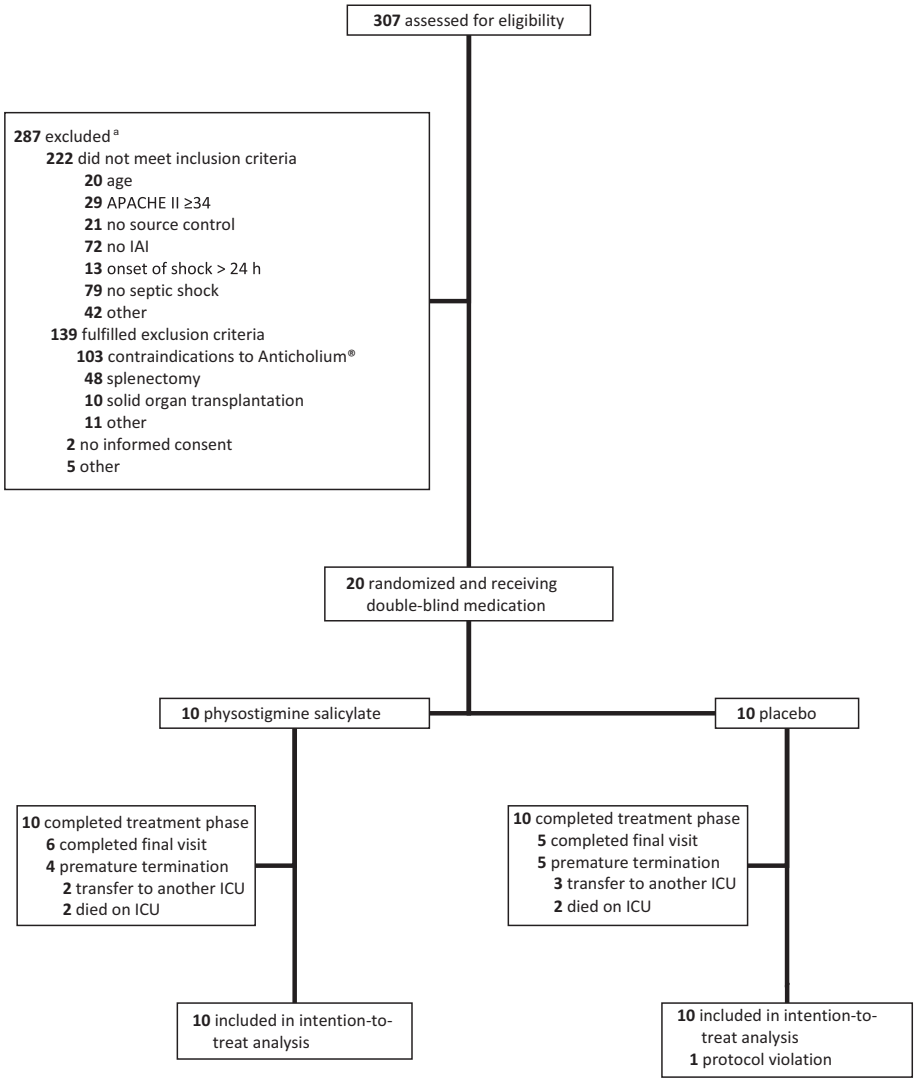

Fig. 1. Screening and inclusion process for participants Between September 2014 and February 2017, 307 patients were assessed for eligibility. Among these, 287 patients violated one or more eligibility criteria. Twenty patients were enrolled and received IMP for at least 2 days (minimum treatment duration). There were no withdrawals during the study for any reason including harms. [a] Multiple responses per patient possible. Information was retrieved from screening logs and may not be exhaustive.

Table 1
Demographics, baseline characteristics, severity of disease and supportive therapy at visit 0 (intention-to-treat population).

	Physostigmine salicylate (n = 10)	Placebo (n = 10)	Total (n = 20)	p-Value
Age, mean (SD), y	63 (9)	60 (12)	62 (11)	0.570*[1]
Male sex, No. (%)	9 (90)	8 (80)	17 (85)	0.531*[2]
Body weight, mean (SD), kg	99.1 (30.7)	76.2 (19.2)	87.7 (27.6)	0.054*[1]
BMI, mean (SD), kg/m^2	30.9 (7.8)	25.3 (5.6)	28.1 (7.2)	0.076*[1]
SOFA score, mean (SD)[a]	12.0 (2.4)	13.9 (3.7)	13.0 (3.2)	0.286*[1]
SOFA subscores, median (IQR)				
Cardiovascular	4 (4–4)	4 (4–4)	4 (4–4)	1.000*[1]
Respiratory	3 (3–4)	3 (3–3)	3 (3–4)	0.720*[1]
Coagulation	0 (0–0)	1 (0–2)	0 (0–2)	0.109*[1]
Renal	1 (0–1)	2 (1–4)	1 (0–2)	0.125*[1]
Hepatic	1 (0–1)	1 (0–1)	1 (0–1)	0.934*[1]
Central nervous system[b]	3 (3–3)	3 (3–3)	3 (3–3)	1.000*[1]
Central nervous system[c]	4 (4–4)	4 (4–4)	4 (4–4)	1.000*[1]
GCS (suspected), mean (SD)	7.4 (3.4)	8.1 (2.4)	7.8 (2.9)	0.116*[1]
APACHE II score, mean (SD)[d]	29.6 (5.3)	30.9 (6.8)	30.3 (6.0)	0.384*[1]
SAPS II score, mean (SD)[e]	61.4 (15.2)	66.4 (12.4)	63.9 (13.7)	0.650*[1]
Lactate, median (IQR), mg/dL [f]	35.0 (21.9–47.9)	39.7 (19.4–69.3)	35.5 (20.7–59.4)	0.678*[1]
Need for vasopressors, No. (%)				
Norepinephrine	10 (100)	10 (100)	20 (100)	
Epinephrine	0 (0)	1 (10)	1 (5)	0.305*[2]
Dobutamine	0 (0)	4 (40)	4 (20)	0.025*[2]
Vasopressin	1 (10)	1 (10)	2 (10)	1.000*[2]
Other [g]	0 (0)	1 (10)	1 (5)	0.305*[2]
Supportive therapy, No. (%)				
Mechanical ventilation	9 (90)	10 (100)	19 (95)	0.305*[2]
RRT	0 (0)	0 (0)	0 (0)	n.a.

Abbreviations: APACHE, Acute Physiology And Chronic Health Evaluation; BMI, Body mass index (calculated as body weight in kilograms divided by height in meters squared); GCS, Glasgow Coma Scale; IQR, Interquartile range; RRT, Renal replacement therapy; SAPS, Simplified Acute Physiology Score; SD, Standard deviation; SOFA, Sequential Organ Failure Assessment.
[a] Scale for SOFA score ranges from 0 to 24, with higher scores indicating greater severity of organ failure; SOFA subscores range from 0 to 4 for each of six organ systems.
[b] Calculated with suspected GCS score if patients were sedated.
[c] Calculated with actual GCS score.
[d] Scale for APACHE II score ranges from 0 to 71, with higher scores indicating greater severity of illness.
[e] Scale for SAPS II score ranges from 0 to 163, with higher scores indicating greater severity of illness.
[a,d,e] Calculated with suspected GCS score. Total score missing, if single subscore missing.
[f] From arterial blood gas analyses.
[g] Here: ephedrine.
*[1] = Wilcoxon test *[2] = Chi^2-test.

Participants' demographics and baseline clinical characteristics are shown in Table 1 (participants' medical history in Supplements). All patients had been diagnosed with diffuse peritonitis during surgery.

3.2. Numbers analyzed

For the primary analysis, all patients of the intention-to-treat population were included, involving all 20 patients who were randomly assigned. The per-protocol population consisted of 10 patients in the physostigmine group and nine patients in the placebo group. Analysis of safety and harms included all randomized patients, again.

Duration of participation in the clinical trial (visit 0 to final visit [day of discharge from ICU] or premature study termination [transfer to another ICU or death]) was 9.5 (6–11) and 14 (5–26) days median (interquartile range [IQR]) in the physostigmine and placebo group, respectively (time and cause of premature study termination in Supplements).

3.3. Outcomes and estimation

3.3.1. Treatment

All participants were treated in accordance with the study protocol; the minimum intended infusion time of 2 days was completed for all subjects. There was no interruption of the initial dose, and no infusion rate deviation (neither in initial dose nor in continuous infusion) at any time; minor interruptions of continuous infusion (maximum 0.7 h) were recorded for five subjects. Total exposure in the physostigmine group (mean ± standard deviation [SD]) was 112.5 ± 27.0 mg, with comparable volumes of sodium chloride in the placebo group (details in Supplements).

3.3.2. Side effects

Administration of physostigmine salicylate using the scheme described in the study protocol was well tolerated and no premature termination of trial medication or modification of infusion rate was necessary. None of the participants showed signs of nausea, vomiting, or changes in airway resistance.

Clinically relevant changes in heart rate (mainly tachycardias) were reported in 20% of all patients during the first 24 h of treatment and were equally distributed with two each in physostigmine and placebo group. In total, six patients in the physostigmine group and three in the placebo group ($p = 0.178$) experienced changes in heart rate during visits 2 to 8, but no treatment was required.

A brief change in blood pressure (hypertension) was observed in one subject in the physostigmine group. Hypertension, classified as an unexpected event in the Summary of Product Characteristics [20], was reported as a suspected unexpected serious adverse reaction, where an arousal/awakening reaction was offered as a possible explanation. However, the hypertensive episode was self-limiting and no treatment was required.

3.3.3. Primary endpoint

In the intention-to-treat population, the mean SOFA score during treatment and subsequent intensive care of up to 14 days was 8.9 ± 2.5 and 11.3 ± 3.6 for physostigmine and placebo group (mean ± SD), respectively (Fig. 2).

Adjusted for age, the difference between SOFA score means did not reach statistical significance (−2.37, 95% CI: −5.43 to 0.70, $p = 0.121$). When baseline SOFA was introduced as covariate (12.0 ± 2.4 and 13.9 ± 3.7 [mean ± SD] for physostigmine and placebo group,

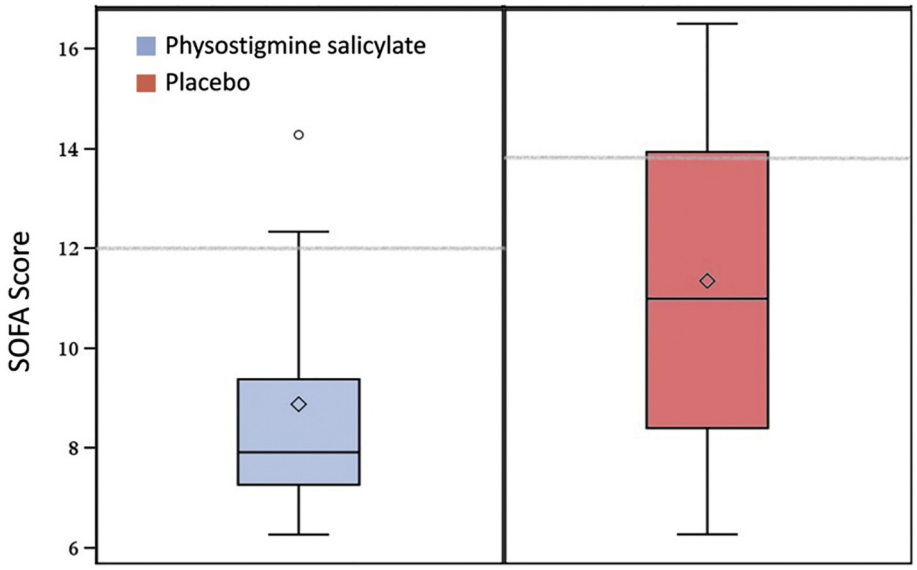

Fig. 2. Mean SOFA score during treatment and subsequent intensive care of up to 14 days (intention-to-treat population) The grey lines indicate baseline SOFA (mean) for each group.

respectively), the difference between means was reduced to −0.99 (95% CI: −3.17 to 1.20, $p = 0.351$).

In the per-protocol population, the mean SOFA score was 8.9 ± 2.5 and 11.1 ± 3.8 for physostigmine and placebo group, respectively (difference between means: −2.07, 95% CI: −5.28 to 1.14, $p = 0.190$, with age as covariate). Subscores of the mean SOFA score during treatment and subsequent intensive care of up to 14 days are given in Table 2.

Fig. 3 depicts the course of disease for both groups. Mean and median SOFA, APACHE II and Simplified Acute Physiology Score (SAPS) II scores may appear higher in the placebo group but remained statistically insignificant (details in Supplements).

3.3.4. Secondary endpoints

3.3.4.1. Supportive therapy. All patients received norepinephrine. Dobutamine remained exclusive to the placebo group (five patients, four of which were treated with dobutamine at baseline; Table 3 and Supplements).

Doses of norepinephrine and mean arterial blood pressure (MAP, details in Supplements) are shown in Fig. 4.

Duration of norepinephrine therapy (any dose) was 4 (4–n.a.) and 5 (5–8) days median (IQR) for physostigmine and placebo group, respectively ($p = 0.778$; n.a.: upper quartile not reached due to continued vasopressor therapy). Duration of septic shock was 4 (3–5) and 5 (4–7) days median (IQR) for physostigmine and placebo group, respectively ($p = 0.528$).

All but one patient, who was ventilated from days 15 to 33 after randomization, were ventilated at baseline and during the treatment phase (Table 3 and Supplements). Duration of mechanical ventilation was 5 (4–10) and 18 (5–n.a.) days median (IQR) in the physostigmine and placebo group, respectively (n.a.: upper quartile not reached due to continued mechanical ventilation). Though mechanical ventilation may appear higher in the placebo group, this remained statistically insignificant ($p = 0.125$; details in Supplements).

Renal replacement therapy was not initiated until visit 3 and was needed for four patients in the placebo group only (one of which had been diagnosed with acute renal failure before). The remaining two patients with pre-existing renal conditions (one per group) did not receive renal replacement therapy during the course of the clinical trial.

3.3.4.2. Further course assessments. Time to cure of the underlying disease and sepsis, and duration of stay in ICU and hospital appeared shorter for the physostigmine group, but did not reach statistical significance (details in Supplements).

By day 30, two and three deaths had been reported for the physostigmine and placebo group, respectively ($p = 0.606$). One additional death per group had been reported at the day 90 assessment ($p = 0.639$). Accordingly, survival was 57 (11–57) and 33 (20−33) days median (IQR) in physostigmine and placebo group, respectively ($p = 0.450$; Fig. 5; causes and time of death in Supplements). No deaths occurred during the treatment phase or the subsequent day (visits 1 to 8, period of adverse event reporting).

Table 2
Subscores of the primary endpoint (mean SOFA score of up to 14 days).

	Physostigmine salicylate (n = 10)	Placebo (n = 10)	Total (n = 20)	p-Value
SOFA subscores, median (IQR)[a]				
Cardiovascular	2.5 (2.3–3.5)	3.3 (2.3–3.8)	3.0 (2.3–3.7)	0.545*[1]
Respiratory	2.5 (2.3–2.8)	2.4 (2.0–2.8)	2.5 (2.0–2.8)	0.733*[1]
Coagulation	0.3 (0–1.3)	0.9 (0–2.7)	0.5 (0–1.6)	0.458*[1]
Renal	0.3 (0–0.6)	1.4 (0.2–2.8)	0.4 (0.2–1.6)	0.129*[1]
Hepatic	0 (0–0.3)	0.4 (0–0.8)	0.1 (0–0.7)	0.362*[1]
Central nervous system[b]	2.0 (1.5–2.2)	2.2 (1.7–2.5)	2.0 (1.6–2.3)	0.449*[1]
Central nervous system[c]	2.5 (1.9–2.9)	2.7 (2.2–3.5)	2.5 (2.0–3.1)	0.385*[1]

Abbreviations: GCS, Glasgow Coma Score; IQR, Interquartile range; SOFA, Sequential Organ Failure Assessment.
*[1] = Wilcoxon test.
[a] Scale for SOFA score ranges from 0 to 24, with higher scores indicating greater severity of organ failure. SOFA subscores range from 0 to 4 for each of the six organ systems.
[b] Calculated with suspected GCS score.
[c] Calculated with actual GCS score.

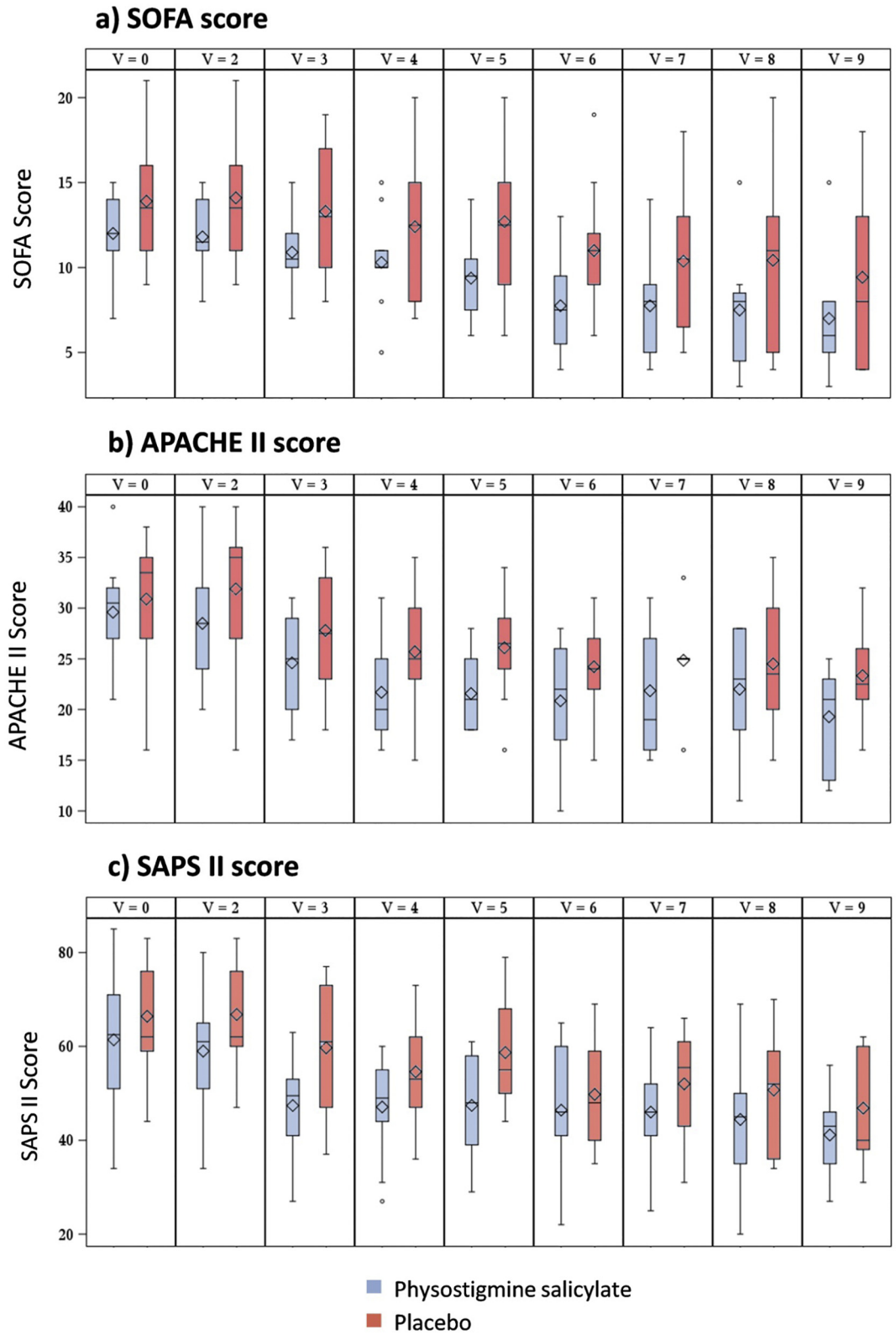

Fig. 3. Course of scores by visit (intention-to-treat population). a) Sequential Organ Failure Assessment (SOFA) score, calculated with suspected Glasgow Coma Score (GCS); b) Acute Physiology And Chronic Health Evaluation (APACHE) II score, calculated with suspected GCS; c) Simplified Acute Physiology Score (SAPS) II score, calculated with suspected GCS.

3.3.4.3. Laboratory values. Leukocyte counts at baseline were 12.2 (8.4–17.8) and 14.0 (5.0–16.1) leukocytes/nL median (IQR) for physostigmine and placebo group, respectively, ($p = 0.910$). Overall leukocyte counts changed little over the course of the trial, including individual increases and decreases. Maximum values of C-reactive protein were significantly higher in the physostigmine group with baseline values of 239.8 (222.2–347.3) and 158.1 (145.6–180.1) mg/L median (IQR) for physostigmine and placebo group, respectively ($p = 0.014$). The difference between groups was statistically significant including visit 3 and C-reactive protein further dropped to 150.4

Table 3
Secondary endpoints: supportive therapy during the course of the clinical trial.

	Physostigmine salicylate (n = 10)	Placebo (n = 10)	Total (n = 20)	p-Value
Need for vasopressors, No. (%)				
Norepinephrine	10 (100)	10 (100)	20 (100)	n.a.
Epinephrine	0 (0)	1 (10)	1 (5)	0.305*2
Dobutamine	0 (0)	5 (50)	5 (25)	0.010*2
Vasopressin	2 (20)	1 (10)	3 (15)	0.531*2
Other	0 (0)	1 (10)	1 (5)	0.305*2
Dose[b] of				
Norepinephrine, mean (SD), μg/kg/min	0.20 (0.11)	0.29 (0.11)	0.24 (0.11)	0.064*1
Epinephrine, mean (SD), μg/kg/min	0.00 (0.00)	0.02 (0.06)	0.01 (0.05)	0.368*1
Dobutamine, mean (SD), μg/kg/min	0.00 (0.00)	0.64 (0.82)	0.32 (0.65)	0.015*1
Vasopressin, mean (SD), units/min	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.584*1
Vasopressor-free days, median (IQR)[a]	3 (0–6)	9 (1–15)	4.5 (0−13)	0.220*1
Mechanical ventilation, No. (%)	10 (100)	10 (100)	20 (100)	n.a.
Ventilator-free days, median (IQR)[a]	2 (1–6)	1 (0–6)	2 (0–6)	0.561*1
RRT, No. (%)	0 (0)	4 (40)	4 (40)	
RRT-free days, median (IQR)[a]		6 (2−12)	6 (2–12)	

Abbreviations: IQR, interquartile range; RRT, renal replacement therapy; SD, standard deviation.
*1 = Wilcoxon test *2 = Chi²-test.
[a] Intervention-free days refer to the entire observation period, i.e. duration of trial participation of each patient (see Supplements).
[b] Mean dose per patient over the entire observation period.

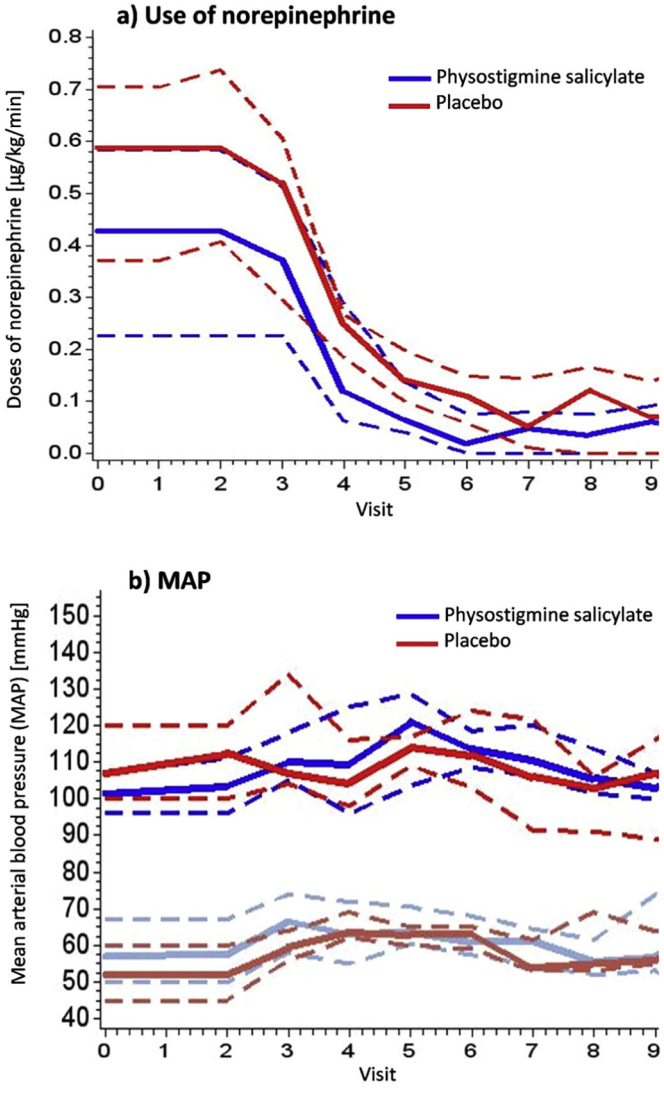

Fig. 4. a) Doses of norepinephrine [μg/kg/min] per visit (intention-to-treat population) b) Mean arterial blood pressure (MAP) [mmHg] per visit (intention-to-treat population), highest and lowest value of MAP documented in previous 24 h (before each visit). Solid lines represent median values, dotted lines represent upper and lower quartiles.

(47.2–183.8) and 104.2 (64.1–140.2) mg/L median (IQR), respectively, at visit 9 ($p = 0.701$).

Procalcitonin levels were 24.1 (12.8–31.4) and 8.3 (1.6–58.8) ng/mL median (IQR) at baseline for physostigmine and placebo group, respectively ($p = 0.860$). For the majority of patients, procalcitonin appeared to increase until visit 2, then rapidly dropped, falling below 1 ng/mL at visit 8. Serum concentrations of the pro-inflammatory cytokine IL-6 varied widely, but the kinetics were comparable to procalcitonin. Baseline IL-6 levels of 5838 (1121–7137) and 3390 (570–11237) pg/mL median (IQR) ($p = 0.734$) decreased to 219 (83–287) and 107 (40–123) pg/mL at visit 8 for physostigmine and placebo group, respectively ($p = 0.272$).

3.3.4.4. Electrocardiogram analyses. Electrocardiogram studies with pathologic findings were distributed similarly between groups (details in Supplements). Tachycardia and prolonged corrected QT intervals were most frequent immediately after study inclusion.

Mean heart rate, which was 99 ± 16 beats per minute (bpm) in the physostigmine group and 91 ± 17 bpm in the placebo group at visit 0 ($p = 0.344$), had dropped to 83 ± 12 bpm in the physostigmine group after 24 ± 2 h (visit 3) but remained almost unchanged in the placebo group at 94 ± 27 bpm ($p = 0.570$). At visit 8, heart rate in the physostigmine group had further stabilized (73 ± 9 bpm) but little change was observed for the placebo group (88 ± 13 bpm, $p = 0.018$). All electrocardiogram studies performed at the final visit ($n = 11$) showed normal heart rates at discharge from the ICU.

3.4. Harms

In total, 331 adverse events and 38 serious adverse events were observed, the majority of which were graded as mild or moderate in severity. Serious adverse events included blood and lymphatic system disorders (nine events) and cardiac disorders (six events). Ten events were categorized as investigations, mainly laboratory test results (vancomycin drug level, transaminases and bilirubin were increased).

Most adverse events (92.7%) and serious adverse events (84.2%) were classified as not related to IMP administration. Several cases of mild or moderate tachycardia and increased blood glucose level were observed in both groups. The only serious adverse events reported as possibly drug-related were tachycardia (1 case), hypertension (1 case), and tachyarrhythmia absoluta (3 cases) in the physostigmine group. Tachyarrhythmia absoluta also occurred in the placebo group (1 case). Two patients experiencing an episode of tachyarrhythmia absoluta had been diagnosed with atrial fibrillation previously.

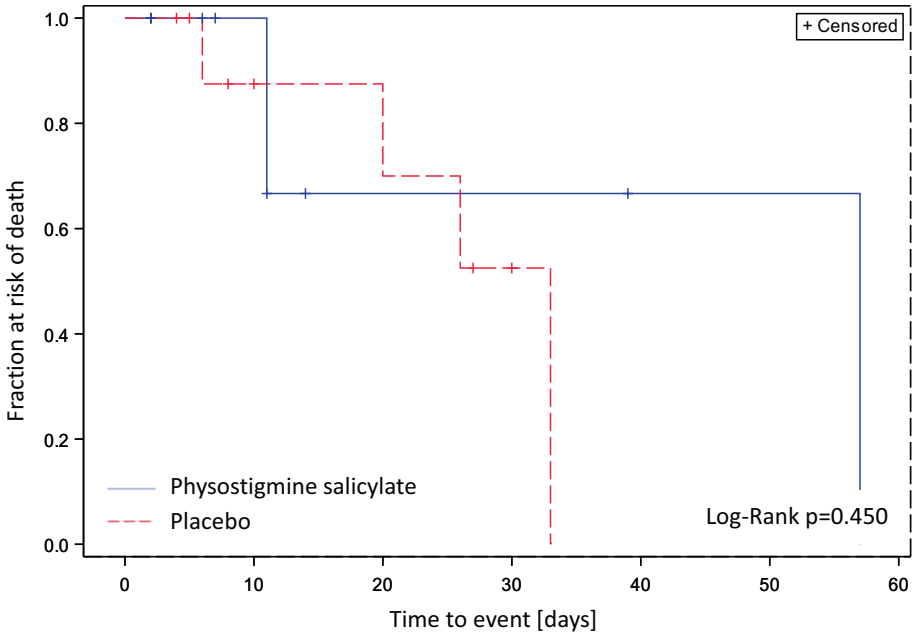

Fig. 5. Survival analysis, i.e. time from randomization to death (intention-to treat population).

4. Discussion

Anticholium® per Se was a randomized, double-blind, placebo-controlled, monocentric pilot trial to assess whether the preclinically described pharmacological activation of the cholinergic anti-inflammatory pathway by administration of physostigmine salicylate may be feasible and safe when transferred to a clinical setting, exploring a new adjunctive therapy in patients with septic shock.

Treatment with physostigmine salicylate as an initial bolus and subsequent continuous infusion for up to five days was found to be practicable, and IMP was well tolerated. Prior to this study, concerns had been raised about the administration scheme, particularly the initial bolus infusion which was at a rate exceeding that used in general practice. Besides nausea and vomiting [20,21], serious cardiac events (bradycardia, asystole) and neurotoxicity (seizures) associated with rapid infusion of physostigmine salicylate had been reported from its use as an antidote for anticholinergic poisoning [22,23]. Pharmacologic data on continuous infusion of physostigmine salicylate are limited [24-26].

However, there were no cases of nausea and vomiting in sedated participants in the present study. Changes in heart rate (tachycardias) were more common in the physostigmine group but had no statistical or clinical significance. These episodes with elevated heart rate under physostigmine salicylate may be explained by central stimulation of epinephrine release from the adrenal gland, likely counteracting muscarinic effects on heart rate, resulting in tachycardia [27,28]. Brief self-limiting episodes of elevated heart rate were observed in our study, even in patients with previously diagnosed tachycardia, which is consistent with published reports [27,28].

The mean SOFA score (primary endpoint) of patients with septic shock due to intra-abdominal infection (assessed over a period of up to 14 days) was not significantly different between physostigmine and placebo group, possibly due to the limited number of participants. The numeric difference may be explained by slight differences in severity of illness at baseline, not uncommon in studies with small sample sizes [29]. On the one hand, prior to treatment commencement with IMP, dobutamine was needed for patients in the placebo group only, which may be indicative of greater severity of illness. On the other hand, C-reactive protein was significantly higher in the physostigmine group, possibly indicating an elevated inflammatory state. However, no difference was demonstrated by SOFA, APACHE II or SAPS II scores at baseline, and groups were comparable with regard to median number of SIRS criteria met, an independent predictor of mortality [30].

Along with the observation that the required doses of norepinephrine may appear lower for patients under treatment with physostigmine salicylate, we found that mean heart rate was reduced by 26 bpm in the physostigmine group, accordingly. This observation is consistent with previous studies in mice [16] indicating less hemodynamic instability [16]. The postulated anti-inflammatory mechanism of cholinesterase inhibitors such as physostigmine salicylate, is to activate a neuroimmune circuit by increasing acetylcholine thus stimulating nicotinic receptors on macrophages, resulting in suppression of cytokine release [11,14-16]. Polymicrobial sepsis, modelled in C57BL/6 mice by cecal ligation and puncture, was associated with reduced concentrations of circulating TNF-α, IL-1β and IL-6 in animals treated with intraperitoneal injections of nicotine, physostigmine salicylate or neostigmine, compared with placebo. Of note, treatment with cholinesterase inhibitors additionally diminished the decrease in blood pressure following infection, and protected the mice against lethal progression from sepsis to severe sepsis and septic shock [16], which may be supported by our finding of a potential beneficial hemodynamic effect of treatment with physostigmine salicylate. Further investigation is required to determine the consistency of this observation.

Inflammatory response to infection, as derived from leukocyte count, C-reactive protein, procalcitonin, and IL-6, did not clearly indicate differences between physostigmine and placebo group. Unlike the rather unspecific leukocyte count, C-reactive protein and procalcitonin dropped almost simultaneously in the first days following initiation of treatment, as did IL-6. Secreted by macrophages and T-cells during early immune response, IL-6 acts as an endogenous pyrogen, up-regulating acute phase proteins and release of additional mediators from macrophages [31]. IL-6 levels may increase from a low pg/mL (normal) to a μg/mL range in septic shock [32]; high levels of IL-6 are associated with increased mortality [33,34]. In this study, remarkably high IL-6 levels were found in two cases, but the difference between the groups remained insignificant, which is in contrast to previous findings in animal models [11,16].

Analysis of survival did not reach statistical significance, which is to be expected given the small sample size. Mortality rates were

lower than anticipated from current literature, with up to 59% for patients with septic shock [6], and estimates from baseline SOFA [35,36], APACHE II [37] and SAPS II scores [38]. Overall placebo group mortality in our study (40%) was found to be consistent with the mean rate of about 39% in recent clinical trials [39,40], although these comparisons are impeded by heterogeneous eligibility criteria and inadequate data reporting in studies [39,40]. It is not surprising that mortality rates in clinical trials are lower than those observed in clinical practice, as severity of illness and comorbidities have been shown to differ profoundly between eligible and ineligible patients [41].

Cautious adverse event reporting was used in this study, pathological laboratory values were included regardless of the underlying disease, which may explain the great number of events reported. However, the majority of adverse events were graded as mild or moderate, classified as not related to IMP administration by the investigators, and were deemed to be a result of the complexity and severity of septic diseases found in the participants. Incidence of adverse events was comparable between groups, although more cardiac events occurred under physostigmine salicylate treatment. The cases of tachycardia and tachyarrhythmia absoluta had completely recovered and resolved by the end of the observation period.

Major strengths of this study include the randomization, blinding, and high adherence to the protocol. The SOFA score is readily available, widely used and clinically accepted, and correlates well with ICU outcome [36]. Mean SOFA score has been previously described as a valid primary endpoint [42], even if maximum SOFA, fixed-day SOFA or delta SOFA are also frequently reported in clinical trials [39,43]. Of note, a potential effect may be underestimated by mean SOFA, averaging values over up to 14 days, but the course of daily SOFA scores was in line with mean SOFA findings. Unfortunately, mean follow-up was shorter than anticipated because of premature study termination in several participants (primarily caused by transfer to another ICU). The trial protocol limited follow-up of participants to their stay in ICU for practicability and to maintain data integrity. Among a variety of secondary endpoints, mortality rates were lower than expected, which may be explained by the exclusion of patients with APACHE II scores ≥34 at screening, although this has been previously reported for clinical trials [44,45].

Even though randomization and stringent eligibility criteria were employed and explorative tests at baseline were statistically insignificant, an imbalance may have existed in the study groups. This may be because the sample size was small and did not allow adjustment for all possible confounders. Individual patients' characteristics including previous, concomitant, and specific underlying diseases with their inherent courses of illness may have affected statistics. However, sepsis may not be a valid entity [46], because critically ill patients are heterogeneous [47,48].

5. Conclusions

Septic shock remains a major challenge, even in modern intensive care, and new approaches to treatment are required, such as investigated by the clinical trial described here. Administration of physostigmine salicylate as an initial bolus followed by continuous infusion for up to five days was feasible and safe. The primary endpoint, mean SOFA score, was not significantly different between groups. However, our results support previous studies in mice hypothesizing that physostigmine salicylate treatment may result in less hemodynamic instability during septic shock.

Further studies are needed to better understand the cholinergic anti-inflammatory pathway and the complex underlying mechanisms. The results reported here justify further studies to assess the effect of physostigmine salicylate on recovery from septic shock in more patients.

Declarations

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Competing interests

NP, TBru, ML, JM, TBre, JL, PK, THT, SS and JBZ declare no competing interests. MAW and SH have served as speakers for and SH has received tangible means and travel reimbursement from Dr. Franz Köhler Chemie GmbH. The funders had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Authors' contributions.

NP conducted the study and made substantial and crucial contributions to the statistical analysis plan and interpretation of data for the work, respectively. TBru drafted the statistical analysis plan and syntax and made substantial contributions to the interpretation of the data for the work. ML was responsible for project management. JM acted as deputy investigator. TBre and JL actively supported NP and JBZ on site (recruitment). PK supported NP and JBZ with logistics. TBre, JL, PK and SS patiently revised the manuscript, significantly improving it. THT and SS made substantial contributions to the design of the study. MAW acted as medical coordinator and SH made substantial contributions to the conception of the work. JBZ acted as investigator and sponsor representative, assuming responsibility for the commissioning, organization and financing; NP and he drafted the manuscript. All other authors revised the manuscript and have given final approval of the version to be published.

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Appendix A. Supplementary data

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