

The ATHOS-3 trial, angiotensin II and *The Three Musketeers*

Rinaldo Bellomo and Andrew Hilton

Hypotension is the defining haemodynamic marker of shock in general, and of septic shock in particular, and is the target of therapeutic interventions worldwide.¹ The aim of such interventions is to restore blood pressure deemed sufficient to maintain vital organ perfusion, while interventions treating the underlying cause of shock are being implemented. In the case of septic shock or other forms of inflammatory vasodilatory shock, interventions applied to restore adequate blood pressure typically include rapid administration of intravenous fluid (fluid bolus therapy [FBT]), administration of a vasopressor drug, or both.¹

The rapid administration of fluid in septic shock is based on the notion that vasodilatation and increased capillary leakiness lead to intravascular fluid depletion, which in turn causes decreased cardiac output. This, again in turn, is thought to be partly responsible for the hypotension observed and for most of the decreased tissue perfusion. Such decreased tissue perfusion is inferred from the presence of hyperlactataemia, oliguria, decreased renal function, altered mental state, decreased capillary refill time and mottled skin.² This paradigm is attractive and simple. However, like all simple explanations for complex phenomena, it is open to challenge. In particular, the native (pre-resuscitation) cardiac output in septic shock may actually be preserved or even supranormal;³ the elevated lactate may represent the metabolic expression of an increase in adrenergic state;⁴ oliguria and loss of renal function may represent intrarenal shunting⁵ rather than decreased perfusion; and, similarly, the cerebral and cutaneous manifestation of shock may also represent microvascular shunting. All these conditions may well be unresponsive to FBT. In the simple case of hypotension, the evidence is that any effect of FBT is minimal, dissipates rapidly and is typically undetectable 1 hour after a given bolus.⁶⁻⁸

Faced with such lack of efficacy, in patients for whom hypotension is considered too severe to be tolerated, clinicians typically turn to the use of vasopressors. Vasopressors are reliably efficacious in restoring blood pressure and certainly much more so than FBT.⁹⁻¹² Among clinically available vasopressors, three agents, like the formidable three musketeers of Alexandre Dumas' book, have dominated the therapeutic armamentarium: epinephrine (adrenaline), norepinephrine (noradrenaline) and vasopressin (arginine vasopressin). These agents are used by clinicians, alone or in combination, to maintain macro-haemodynamic stability. This stability is defined by

adequate cardiac output and mean arterial pressure and, it is hoped, evidence of stable or improving vital organ function and markers of microvascular recovery.

In *The Three Musketeers*, each of the three, however skilled with the sword, had serious character flaws; and in the end there were four. Epinephrine, like the impetuous d'Artagnan, can deliver combined alpha and beta effects promptly. However, the effects are accompanied by tachycardia, hyperglycaemia, hyperlactataemia, increased risk of arrhythmia and potentially adverse effects on renal perfusion.^{13,14} Norepinephrine, like the muscular Porthos, can deliver blood pressure control under almost all circumstances, but it may induce myocardial cell band necrosis, increase the risk of atrial fibrillation, induce cutaneous vasoconstriction and digital hypoperfusion, and may decrease renal medullary oxygenation.^{15,16} Vasopressin, like the sophisticated Aramis, typically reflects a greater level of nuanced haemodynamic management delivered in the intensive care unit. However, it also carries risks and limitations, such as its profound splanchnic vasoconstrictive effects and the limited ability to titrate it beyond an upper fixed-dose range. Despite promising early evidence, a large randomised controlled trial testing its effectiveness failed to identify a beneficial effect on patient-centred outcomes in septic shock.¹²

In this context, the Angiotensin II for the Treatment of High-Output Shock 3 (ATHOS-3) trial, named after the oldest and most secretive remaining musketeer, matters a great deal. Recruitment for the trial was recently completed and involves about 75 ICUs worldwide, including multiple sites in Australia and New Zealand. The ATHOS-3 trial introduces another powerful vasopressor agent, angiotensin II (ANG II). In the trial, as described in the protocol and statistical analysis plan and as reported in this issue of the Journal,¹⁷ ANG II was compared with placebo as an adjunctive vasopressor in patients requiring a catecholamine dose > 0.2 mg/kg/min. Started at 20 ng/kg/min, the dose was titrated according to a strict protocol with a haemodynamic primary endpoint, as prescribed by the US Food and Drug Administration (FDA), and with change in Sequential Organ Failure Assessment score as a secondary outcome.

The application of ANG II to the management of vasodilatory shock is physiologically logical. ANG II is one of the four vasoconstrictive hormones secreted in response to shock in order to defend blood pressure (epinephrine, norepinephrine, vasopressin and ANG II). It is also not new;

previous uncontrolled studies have reported the efficacy of ANG II as a vasopressor and have not reported unexpected safety concerns.⁹⁻¹¹ Moreover, animal studies have reported efficacy in restoring urinary output and creatinine clearance in a model of septic shock.¹⁸ Finally, assessment of renal bioenergetics during its infusion has dispelled concerns that its vasoconstrictive effect on the efferent glomerular arteriole would induce marked renal tissue hypoxia.¹⁹

What is new is that, if the ATHOS-3 trial shows both safety and efficacy, it will provide the ICU community with likely FDA approval for the use of ANG II in vasodilatory shock. For the Australian and New Zealand community, Therapeutic Goods Administration approval would likely then follow and make the agent available to local clinicians. Such availability may lead to “balanced” vasopressor therapy, which, by combining at least three of the vasoactive musketeers (ANG II, vasopressin and norepinephrine) will attempt to simulate the typical physiological response to severe vasodilatation and hypotension. This balanced vasopressor therapy might also compensate for its limited ability to deal with severe overwhelming vasodilatation, maximise efficacy and minimise toxicity. Combined with appropriate inotropic support in cases of myocardial depression, this approach may prove physiologically optimal. If so, a true phase III trial with patient-centred outcomes may well follow. Like the fate of Cardinal Richelieu at the end of *The Three Musketeers*, religious use of FBT will be challenged by the need to treat the three, and now four, vasopressors with greater respect.

Competing interests

None declared.

Author details

Rinaldo Bellomo, Professor of Intensive Care¹

Andrew Hilton, Associate Professor and Staff Specialist²

1 School of Medicine, University of Melbourne, Melbourne, VIC, Australia.

2 Department of Intensive Care, Austin Hospital, Melbourne, VIC, Australia.

Correspondence: rinaldo.bellomo@austin.org.au

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Angiotensin II for the Treatment of High-Output Shock 3 (ATHOS-3): protocol for a phase III, double-blind, randomised controlled trial

Lakhmir S Chawla, James A Russell, Sean M Bagshaw, Andrew D Shaw, Stuart L Goldstein, Mitchell P Fink and George F Tidmarsh

Shock is common among critically ill patients. Although shock can occur without hypotension, severe shock is almost always associated with hypotension.¹ Severe shock is described as persistent hypotension requiring vasopressors to maintain a mean arterial pressure (MAP) of 65 mmHg and a serum lactate concentration of > 2 mmol/L despite adequate volume resuscitation. In patients with severe shock requiring treatment with high doses of vasopressors (eg, catecholamines and vasopressin), their use is associated with poor outcomes.² Catecholamine-resistant hypotension (CRH) is characterised by inadequate response to usual doses of standard-of-care (SOC) vasopressor therapy, and increased mortality.³ In general, health care professionals use two classes of vasopressors in hypotension: catecholamines and vasopressin-like peptides. However, humans physiologically employ three classes of vasopressors (catecholamines, vasopressin and angiotensins) to maintain blood pressure. Thus, for patients with severe hypotension, the addition of angiotensin II (ANGII) may be beneficial.

ANGII, an octapeptide hormone, is a potent vasopressor that induces vasoconstriction through activation of the ANGII type 1 receptor (AT1R) and functions as an integral component of the renin–angiotensin–aldosterone system.⁴ AT1R activates a G-coupled protein pathway that subsequently activates phospholipase C, inducing vasoconstriction.

Synthetic ANGII shows effective vasopressor activity and has been used to improve MAP in patients with CRH.^{5–9} In a recent pilot study of synthetic human ANGII in patients with CRH, ANGII increased MAP and decreased catecholamine use, both of which could be beneficial.¹⁰ Our study is designed to determine if LJPC-501 (synthetic human ANGII acetate) could increase MAP in patients with CRH. We hypothesise that a greater proportion of patients with CRH treated with ANGII than those treated with placebo will achieve an increase in MAP.

Study rationale

There is an ongoing need for more effective approaches to CRH therapy.¹¹ The Angiotensin II for the Treatment of High-Output Shock 3 (ATHOS-3) trial was designed to compare

ABSTRACT

Objective: Catecholamine-resistant hypotension (CRH) is characterised by inadequate response to standard doses of vasopressors, and increased mortality. Our Angiotensin II for the Treatment of High-Output Shock 3 (ATHOS-3) trial compares the efficacy and safety of angiotensin II (ANGII) versus placebo in CRH.

Design, setting and participants: A phase III, multicentre, randomised, placebo-controlled trial of LJPC-501 (synthetic ANGII) for CRH in up to 120 intensive care units. We have set a target of 300 critically ill patients with CRH receiving standard-of-care (SOC) vasopressor therapy (ie, catecholamine dose >0.2 µg/kg/min for 6–48 hours to maintain a mean arterial pressure [MAP] of 55–70 mmHg). Calculation of a norepinephrine-equivalent vasopressor dose is critical to determining patient eligibility, as ANGII will supplement ongoing vasopressor therapy.

Interventions: Stable patients will be randomised 1:1 to SOC vasopressor plus continuous intravenous infusion of ANGII or placebo for 48 hours, with an aim of achieving MAP of 75 mmHg for the first 3 hours. ANGII (initiated at 20 ng/kg/min) will be titrated according to pre-specified guidelines until 48 hours, with patients followed until Day 7. Frequent vital sign and haemodynamic monitoring will support ANGII titration, safety monitoring and efficacy assessments.

Main outcome measures: The primary efficacy endpoint is MAP ≥75 mmHg or an increase of ≥10 mmHg at treatment Hour 3. Secondary endpoints include change in total and cardiovascular Sequential Organ Failure Assessment scores over 48 hours, and safety data.

Conclusion: Our study will investigate the utility of adding ANGII to current SOC vasopressor options to increase the efficacy and safety of CRH therapy.

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the efficacy and safety of intravenous ANGII with placebo in patients with CRH. The primary endpoint is based on MAP at 3 hours after study drug initiation during maintenance of vasopressor therapy.

Methods

Design

This is a multicentre, randomised, double-blind, placebo-controlled trial of LJPC-501 for improvement of MAP (ClinicalTrials.gov/NCT02338843).

Setting

The trial has been initiated at about 75 investigational sites (intensive care units) in the United States, Canada, Australia, New Zealand, the United Kingdom, Belgium, Finland, France and Germany.

Table 1. Patient enrolment criteria

Inclusion criteria

- ≥ 18 years old with catecholamine-resistant hypotension*
- Central venous access and an arterial line†
- Indwelling urinary catheter‡
- Received ≥ 25 mL/kg of crystalloid or colloid equivalent over previous 24 hours and had adequate volume resuscitation†
- Clinical features of high-output shock§
- Written informed consent
- Exclusion criteria
- Burns covering > 20% of total body surface area
- Cardiovascular SOFA score ≤ 3
- Acute occlusive coronary syndrome
- Receiving VA ECMO; receiving VV ECMO for < 12 hours
- Current bronchospasm or history of asthma, Raynaud phenomenon, systemic sclerosis or vasospastic disease
- Current liver failure¶
- Acute or past mesenteric ischaemia
- Current, past or suspected aortic dissection or abdominal aortic aneurysm
- Requires hydrocortisone > 500 mg/day or equivalent glucocorticoid medication**
- Expected to die within 12 hours
- Active bleeding, need for > 4 units packed red blood cells, or haemoglobin level < 7 g/dL
- Contraindication for serial blood sampling
- Absolute neutrophil count < 1000 cells/mm³
- Known allergy to mannitol
- Current participation in trial of another interventional drug or device
- Pregnant at screening

SOFA = Sequential Organ Failure Assessment. VA = venoarterial. ECMO = extracorporeal membrane oxygenation. VV = venovenous. * Defined as requiring a total sum vasopressor dose of > 0.2 µg/kg/min for 6 to 48 hours to maintain a mean arterial pressure of 55–70 mmHg (see Table 3 for conversion to norepinephrine equivalent). † Must remain present for first 48 hours of the study. ‡ Investigator-assessed. § Central venous oxygen saturation > 70% (measured by oximetry catheter or central venous blood gas) and central venous pressure > 8 mmHg or a cardiac index > 2.3 L/min/m². ¶ Model for End-Stage Liver Disease score ≥ 30. ** See Appendix 2 (online at cicm.org.au/Resources/Publications/Journal) for glucocorticoid-equivalent medication dosing.

Population

We will use trial inclusion criteria (Table 1) to identify a population of about 300 critically ill adults with severe high-output shock, exhibiting CRH, who require high-dose vasopressors. All six inclusion criteria must be fulfilled at the time of screening.

Patient screening

Screening for eligibility will be conducted within the 48 hours immediately before study drug initiation; written informed consent to participate in the study must be provided by each patient or their surrogate before screening. Because study drug is being added to ongoing SOC vasopressor therapy, we have calculated norepinephrine-equivalent vasopressor doses (Table 2) to standardise baseline vasopressor therapy and determine patient eligibility. Eligible patients will be stratified and randomly assigned to treatment (details below). All assessments performed during screening are detailed in Table 3 and Appendix 1 (online at cicm.org.au/Resources/Publications/Journal).

Ethics and consent

The study is being conducted in accordance with good clinical practice, following applicable local regulations and with the ethical principles described in the Declaration of Helsinki. It has been approved by the appropriate ethics committee or institutional review board at each study centre. Patients or their legal surrogate must be willing and able to provide written informed consent and comply with all protocol requirements.

Treatment assignment

Following confirmation of eligibility and consent, central randomisation will be used to assign patients to receive ANGII or placebo (Figure 1). Blocked randomisation within strata will use a 1:1 schedule of placebo:active comparator, with screening MAP (< 65 mmHg and ≥ 65 mmHg) and

Table 2. Conversion of vasopressors to norepinephrine equivalent*

Drug equivalent	Dose	Norepinephrine
Epinephrine†	0.1 µg/kg/min	0.1 µg/kg/min
Norepinephrine†	0.1 µg/kg/min	0.1 µg/kg/min
Dopamine†	15 µg/kg/min	0.1 µg/kg/min
Phenylephrine‡	1.0 µg/kg/min	0.1 µg/kg/min
Vasopressin§	0.04 U/min	0.1 µg/kg/min

* Conversion scale developed by the authors (L C, J R and G T).

† Conversion based on the cardiovascular Sequential Organ Failure Assessment score. ‡ Conversion based on medical literature.

§ Conversion developed (by J R) using Vasopressin and Septic Shock Trial data set.¹¹

Acute Physiology and Chronic Health Evaluation (APACHE) II score (≤ 30 , 31–40 and ≥ 41) as stratification variables.

Dosing regimen

Before study drug initiation, patients will be re-assessed to confirm that they have had adequate volume resuscitation. Patients may receive up to 750 mL of intravenous fluids during the first 3 hours of the study if required for safety reasons.

Doses of SOC vasopressors will be optimised before initiating the study drug, using criteria for conversion of catecholamine and vasopressin dosing to norepinephrine equivalents (Table 2), and kept constant during the first 3 hours of study drug treatment. Inotropes (eg, dobutamine and milrinone) may be titrated during the first 3 hours. Study drug will be titrated based on patient MAP (Table 4). If safety requirements dictate, SOC vasopressor doses may also be titrated to maintain MAP. If a patient’s SOC doses are increased in the first 3 hours, they are classified as having failed treatment but they may continue to receive study drug treatment. Exceptions to failure include epinephrine and dopamine given in the inotropic range.¹²

Drip weight (the patient bodyweight at which SOC vasopressors are being dosed) will be used to calculate study drug dosing, beginning at 20 ng/kg/min. Patients assigned to placebo will receive volume-matched infusion increments of 0.9% NaCl solution. Dose titrations based on MAP can occur as frequently as every 5 minutes, and MAP will be determined as the average of three values obtained ≥ 1 minute apart. Dose titration intervals (Table 4) and permissible dosing adjustments based on MAP at each assessment time point (the key indicator for dosing decisions) are pre-specified for Hours 0–3, 3–48 and, if needed, 48–168.

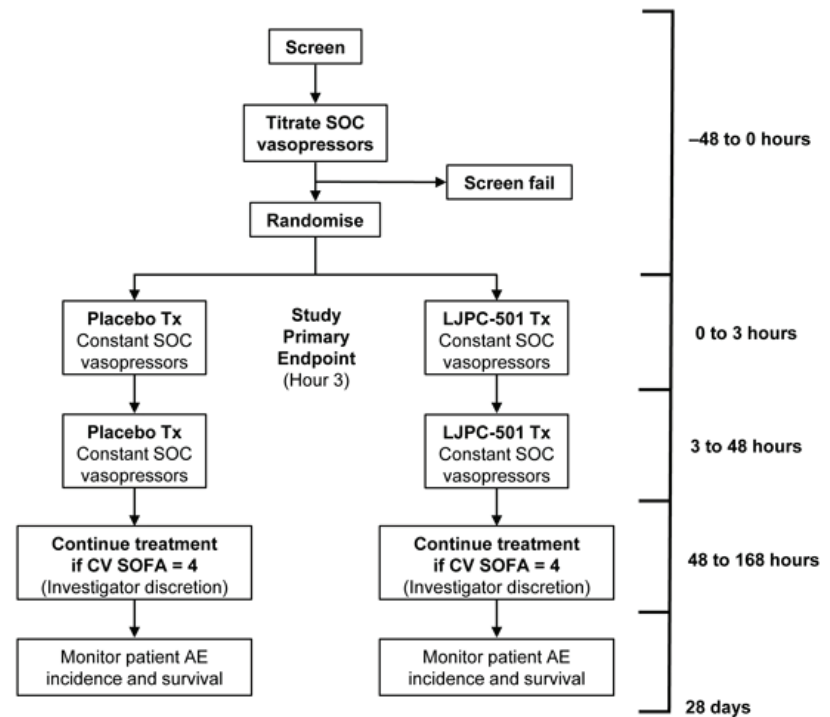
Study drug administration will be initiated at Day 1, Hour 0, and continue until Hour 48, at which time it will be down-titrated and terminated. Subsequently, if a patient’s cardiovascular (CV) Sequential Organ Failure Assessment (SOFA) score is 4 after discontinuing the study drug, dosing may resume within 3 hours and continue

Table 3. Patient assessments at screening

- Titrate SOC vasopressors and document SOFA score
- Obtain a complete medical history*
- Document current medications†
- Perform a limited physical examination‡
- Document MAP hourly over a 6-hour period during SOC vasopressor titration
- Obtain a 12-lead electrocardiogram
- Determine central venous pressure and assess cardiac output when feasible
- Obtain a chest x-ray
- Collect blood samples for analysis§
- Determine arterial blood gases for respiratory component (P_{aO_2}/F_{iO_2}) of SOFA score
- Determine blood oxygenation (alveolar–arterial gradient or P_{aO_2} , depending on F_{iO_2}) and arterial pH for APACHE II score
- Record ventilator settings (tidal volume, PEEP) for patients on mechanical ventilation
- Perform Glasgow Coma Scale assessment to calculate nervous system component of SOFA score, preferably during a sedation holiday
- Collect urine for routine dipstick urinalysis
- Document hourly urine output in the 6 hours before initiation of study drug

SOC = standard-of-care. SOFA = Sequential Organ Failure Assessment. MAP = mean arterial pressure. APACHE II = Acute Physiology and Chronic Health Evaluation II. PEEP = positive end-expiratory pressure. * Including demographic data, catecholamine-resistant hypotension diagnosis, surgical history and concurrent medical conditions. † Including antihypertensive medications taken within 7 days, vasopressors and procedures within 2 days of study drug administration. ‡ Including bodyweight, height and vital signs. § Including clinical chemical and haematological tests with differential blood counts, plasma angiotensin I and II concentrations, and samples to bank for possible later analyses. Total bilirubin levels, serum creatinine levels and platelet counts will be used to calculate the liver, renal and coagulation components of the SOFA score, respectively.

Figure 1. Study design



SOC = standard-of-care. Tx = treatment. CV SOFA = cardiovascular Sequential Organ Failure Assessment score. AE = adverse event.

Table 4. Study drug titration schema

Mean arterial pressure (mmHg)	Initial dose (ng/kg/min)	Titration interval (min)	Dose titration (ng/kg/min)	Max. dose (ng/kg/min)	Min. dose (ng/kg/min)
Hours 0–3 (target MAP, ≥ 75 mmHg)					
≤ 59	20	5	Increase to 80 then by increments of 20*	200	2.5
60–74	20	15	Increase by 10	200	2.5
75–84	na	15	Maintain dose	200	2.5
≥ 85	na	5	Decrease by 10 [†]	200	2.5 [‡]
Hours 3–48 (target MAP, 65–70 mmHg)					
≤ 59	–	5	Increase to 40	40	2.5
60–64	–	15	Increase by 10	40	2.5
65–70	–	15	Maintain dose [§]	40	2.5
≥ 70	–	15	Decrease by 10 [¶]	40	2.5**
Hour 48 to Day 7 (target MAP, 65–70 mmHg)					
≤ 59	–	5	Increase to 40	40	2.5
60–64	–	15	Increase by 10	40	2.5
65–70	–	15	Maintain dose [§]	40	2.5
≥ 70	–	15	Decrease by 10 [¶]	40	2.5**

max. = maximum. min. = minimum. MAP = mean arterial pressure. na = not applicable (patients not eligible for study participation). SOC = standard-of-care. * Dosing schema may be modified by consensus of data and safety monitoring board to as low as 60 ng/kg/min and as high as 120 ng/kg/min, if deemed necessary for safety purposes. † Once a dose of 10 ng/kg/min is reached, study drug dose may be further reduced by halving each titration until the minimum dose is achieved. ‡ Dosing may be modified to as low as 1.25 ng/kg/min for patients considered hyper-responders (ie, MAP remains ≥ 85 mmHg despite discontinuation of vasopressin and all catecholamines). § If the sum of the norepinephrine and epinephrine doses is ≥ 0.03 but < 0.1 $\mu\text{g/kg/min}$, the study drug dose should be maintained. ¶ If vasopressin is being used, the patient should be weaned off vasopressin first, then titrate SOC vasopressors until the sum of the norepinephrine and epinephrine dose is as low as 0.03 $\mu\text{g/kg/min}$. ** Dosing may be modified to as low as 1.25 ng/kg/min for patients considered hyper-responders (ie, MAP remains ≥ 70 mmHg despite discontinuation of vasopressin and reduction of sum norepinephrine and epinephrine dose to as low as 0.03 $\mu\text{g/kg/min}$).

through to Day 7 (168 hours), at which time it must be titrated off.

Assessments

Pre-defined on-treatment and post-treatment assessments (Appendix 1) will include very frequent monitoring of vital signs and haemodynamic parameters to support study drug titrations and assessments of therapeutic responses.

Study endpoints

Primary efficacy endpoint

The primary efficacy endpoint is MAP response, defined as a mean MAP of ≥ 75 mmHg or a ≥ 10 mmHg increase in MAP from baseline at treatment Hour 3. For the primary efficacy endpoint, MAP will be measured at 15 minutes before, at, and 15 minutes after the Hour 3 time point on Day 1. No MAP response will be recorded if neither of these endpoints are reached, the patient dies before all three MAP measurements can be taken, or the patient requires an increase in SOC vasopressor dose. In the absence of all three MAP measures, the average of available measures will be used.

Secondary efficacy endpoints

The secondary efficacy endpoints are change in CV SOFA score and total SOFA score between baseline measurement and Hour 48. The total SOFA score is the sum of the six individual SOFA item scores, including CV score. Missing secondary endpoint values at Hour 48 due to death will be assigned the worst possible SOFA score. The last observation will be carried forward for values missing for reasons other than death. We will compare the change in CV SOFA scores between treatment arms using the van Elteren-stratified Wilcoxon rank-sum test, with strata defined by the randomisation strata. The change in total SOFA score will be analysed using the general linear model including all randomisation strata. We will use a norepinephrine-equivalent calculation (Table 3) for all vasopressor dose and CV SOFA score analyses.

Safety monitoring

Safety will be monitored by assessing adverse events (AEs), blood pressure and heart rate, and using routine clinical safety laboratory testing including urinalysis and urine output measurement and testing of blood chemical and haematological parameters (see Appendix 1). All serious and non-serious AEs will be recorded from the first dose

of study drug to Day 7 or the end-of-study visit. Deaths will be captured from the first dose of study drug to Day 28. Serious AEs reportable to regulatory authorities will be captured after Day 7 or at the end of the study through to Day 28. Patients with AEs will be monitored until resolution or stabilisation of the AE. All serious AEs will be reported to the sponsor or its designee and monitored until they are resolved or are clearly determined to be due to a chronic condition or concurrent illness. Reportable safety AEs occurring between study Day 7 and Day 28 will be assessed by a follow-up phone call or chart review to occur about 28 days after study drug initiation.

Vasopressor toxicity

Vasopressor toxicity monitoring will occur throughout the study, and event details will be systematically collected. Safety AEs indicative of vasopressor toxicity include but are not limited to: myocardial infarction and/or ischaemia (beyond simple troponin elevation); relevant dysrhythmias, including atrial fibrillation; re-entrant atrioventricular nodal tachycardia; ventricular tachycardia; cerebral ischaemia; hypoperfusion (eg, digital ischaemia, mesenteric ischaemia, shock liver); renal hypoperfusion and local vasoconstriction or necrosis at or near the infusion site.¹¹

Termination of treatment and/or study participation

Patients may be withdrawn from the study for clinically significant concurrent illness, occurrence of an AE, patient request, major protocol violation, non-compliance, administrative reason, failure to return for follow-up on Day 7, or changes in the patient's condition making further study treatment unacceptable (investigator determination). All study procedures intended for study Day 7 or the end of the study are to be completed at the time of withdrawal. Patients who withdraw after receiving any amount of study drug will be included in the modified intention-to-treat analysis (mITT) population.

Concomitant treatment and supportive care

All patients will be given SOC therapy during the course of the study. Supportive therapy and use of concomitant medications for other medical conditions that are ongoing at baseline (subject to specific protocol requirements) will be permitted during the treatment phase of the study. No other experimental therapy is permitted. All medications and supportive therapies that are administered during the study will be recorded.

Statistical considerations and analysis plan

Sample size estimation

The sample size for our study is based on a hypothesised success rate in the primary efficacy endpoint of 40% in the placebo arm and 60% in the ANGIIL arm. A two-by-two χ^2

test with a two-sided α of 0.05 will have > 90% power to show superiority of ANGIIL over placebo with a sample size of 150 evaluable patients per treatment arm.

Stratification and randomisation

We will use central randomisation. Screening MAP (< 65 mmHg and \geq 65 mmHg) and APACHE II score (\leq 30, 31–40 and \geq 41) will be used as stratification variables. Blocked randomisation within strata with a 1:1 schedule of placebo:active will be used.

Analysis populations

The primary efficacy analysis population will be the mITT population that includes all randomised patients who receive study drug. The per protocol (PP) analysis population will include all mITT patients who were not considered to be major protocol violators. Analyses based on the PP population will be used to support the mITT analyses. The safety population will be all patients who received study drug.

Handling of missing, unused and spurious data

For mITT and PP analyses, primary or secondary endpoint values missing due to death will be imputed as failures. Values will also be imputed when missing due to reasons other than death in the mITT but not the PP analysis population. In the mITT population, the last observed value will be carried forward as a primary imputation method. Multiple imputation, using PROC MI and PROC MIANALYZE (SAS Institute), will be used as a sensitivity analysis for the primary efficacy analysis. No imputation of values for missing data will be performed for the safety population. We will use standard clinical monitoring and data management practices to ensure data integrity.

Statistical methods

We will summarise data by treatment arm and by measurement time using descriptive statistics for continuous and ordinal variables, with 95% confidence intervals. For statistical comparisons of differences between treatment groups, we will use the Wilcoxon rank-sum test or analysis of variance for continuous or ordinal variables and the χ^2 or Fisher exact test for discrete variables.

We will analyse the primary efficacy endpoint using logistic regression. Covariates will include dichotomised baseline MAP, APACHE II score, vasopressor use in the 6 hours before randomisation (with "yes/no" data) and quantity of vasopressor use over the 6 hours before randomisation. A two-tailed α of 0.05 will be used in testing the hypothesis of treatment difference. We will also evaluate the presence of a site effect and site-by-treatment interaction with this model.

We will summarise all AEs, serious AEs and AEs leading to discontinuation, along with their relationship to the study

drug. Summaries will be based on Medical Dictionary for Regulatory Activities terminology (www.meddra.org).

Exploratory analyses

We will conduct exploratory analyses to compare treatment effects in patients stratified according to certain characteristics, including sex, age, baseline MAP, baseline vasopressor dose, baseline APACHE II score, geographical region, recent exposure to angiotensin-converting enzyme (ACE) inhibitors, recent exposure to angiotensin receptor blockers, history of acute respiratory distress syndrome, history of sepsis, baseline plasma angiotensin I and II concentrations and their ratio, and drug sensitivity as measured by study drug dose 30 minutes after initiating the study drug.

Additional exploratory endpoints include mortality at Day 7 and Day 28, analysed by Kaplan–Meier methods and log-rank test; MAP response at Hour 1 and Hour 2, analysed by the same method as MAP response at Hour 3; change in SOFA component scores, analysed by the same method as CV SOFA score analysis; changes in heart rate, vasopressor use, lactate level, serum creatinine level, urine output; and length of time on vasopressor, time on ventilator, and time in the ICU.

Trial oversight

Monitoring and auditing procedures were developed by La Jolla Pharmaceutical Company or its designee in line with good clinical practice and the principles and guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. A site monitor representing the sponsor will conduct periodic site visits to review study source documents and ensure that the investigation is conducted according to protocol design and regulatory requirements. Case report forms will be monitored for completeness, clarity and consistency with source documents. This study will be overseen by a data safety monitoring board, which will be conducted according to the guidelines outlined in a separate, pre-approved charter.

Discussion

Patients with CRH have limited treatment options and, despite some longitudinal improvements in patient survival, mortality remains high for patients with septic shock.¹³ Available rescue therapies, including methylene blue, high-volume haemofiltration and extracorporeal therapies, have not been tested in large, randomised controlled trials, are largely ineffective and are associated with increased AEs.^{14–16} ANGII has shown clinical effectiveness in case reports of patients with CRH.^{5–9} A randomised, placebo-controlled pilot study has also shown that ANGII increased MAP and

decreased catecholamine use.¹⁰ However, because of the limited sample size (10 patients per treatment arm) and short treatment duration (6 hours), statistical significance was not achieved for the primary endpoint and safety was not adequately established. The ATHOS-3 trial is the first phase III trial to compare the efficacy and safety of ANGII with placebo in patients with CRH.

Humans have evolved three classes of vasopressor hormones: catecholamines, vasopressin and angiotensins. Catecholamines and vasopressin, but not angiotensin, are currently approved in most regions for vasoactive support in shock. Reduced renin–angiotensin system activity, whether due to endogenous variability or exogenous pharmaceutical manipulation (inhibition of ACE or AT1R), is associated with increased risk of shock and increased patient mortality in severe sepsis.^{17,18} Further, polymorphism in the AT1R-associated protein gene was associated with increased AT1R protein, decreased blood pressure and increased sepsis mortality,¹⁹ providing additional evidence that inadequate ANGII signalling contributes to poor outcomes.

Our ATHOS-3 trial examines the hypothesis that intensivist access to ANGII in addition to SOC vasopressors could improve blood pressure support and patient outcomes when homeostatic mechanisms fail during an occurrence of shock. Modern advances in the treatment of many complex disease states, such as acquired immune deficiency syndrome, rheumatoid arthritis, psoriasis and refractory hypertension, have relied on combination therapies that target multiple physiological pathways, thereby increasing synergy and avoiding toxicity.^{20–22} Similarly, because both catecholamines and vasopressin have significant toxicities, ANGII may enable synergy by targeting a distinct signalling pathway and facilitate improved outcomes.

Trial status

Recruitment to the trial is complete. This study protocol manuscript was accepted for publication before study completion.

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Competing interests

Lakhmir Chawla and George Tidmarsh are employees of and own stock in La Jolla Pharmaceutical Company, are medical monitors for the study, and have assigned patents relating to the use of angiotensin II for hypotension.

James Russell is an inventor on patents owned by the University of British Columbia that are related to PCSK9 inhibitor(s) and sepsis and related to the use of vasopressin in septic shock. He is a founder, director and shareholder of Cyon Therapeutics, has share options in Leading Biosciences, and is a shareholder in Molecular You Corp. He has received consulting fees from Cubist Pharmaceuticals, Leading Biosciences, Ferring Pharmaceuticals, Grifols, La Jolla Pharmaceutical Company, CytoVale and Asahi Kasei Pharma America. He received an investigator-initiated grant from Grifols that is provided to and administered by the University of British Columbia.

Stuart Goldstein and Andrew Shaw are consultants to La Jolla Pharmaceutical Company to adjudicate the appropriateness of extending treatment with study drug beyond 7 days. Sean Bagshaw is supported by a Canada Research Chair in Critical Care Nephrology. Sean Bagshaw and James Russell were consultants to La Jolla Pharmaceutical Company on the design and development of the ATHOS-3 protocol. They now serve as Chair (J R) and a member (S B) of the ATHOS-3 trial Data Safety Monitoring Board and receive consulting fees for this service.

The trial is funded by La Jolla Pharmaceutical Company.

Author details

Lakhmir S Chawla, Professor,¹ and Chief Medical Officer²

James A Russell, Professor,³ and Principal Investigator⁴

Sean M Bagshaw, Associate Professor⁵

Andrew D Shaw, Executive Vice Chair⁶

Stuart L Goldstein, Professor⁷

Mitchell P Fink, * former Vice Chair for Critical Care⁸

George F Tidmarsh, Chief Executive Officer,² and Consulting Professor⁹

* Posthumously published

1 Department of Medicine, Veterans Affairs Medical Center, Washington, DC, USA.

2 La Jolla Pharmaceutical Company, San Diego, CA, USA.

3 Department of Medicine, University of British Columbia, Vancouver, BC, Canada.

4 Centre for Heart Lung Innovation, St Paul's Hospital, University of British Columbia, Vancouver, BC, Canada.

5 Department of Critical Care Medicine, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada.

6 Department of Anesthesiology, Vanderbilt University Medical Center, Nashville, TN, USA.

7 Center for Acute Care Nephrology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA.

8 Departments of Surgery and Anesthesiology, David Geffen School of Medicine, University of California, Los Angeles, CA, USA.

9 Division of Neonatal and Developmental Medicine, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA.

Correspondence: lchawla@ljpc.com

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