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Ularitide as treatment of refractory ascites in cirrhosis – a study protocol for a randomised trial

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ABSTRACT

INTRODUCTION Ascites is a frequent complication to cirrhosis. When ascites becomes refractory to standard diuretic pharmacotherapy, patients are facing a median survival of less than one year and most likely a need for frequent hospitalisations due to large-volume paracentesis or complications. An unmet need exists for new and improved treatments of refractory ascites and the present study investigates the potential of the natriuretic peptide ularitide for this indication.

METHODS We aim to investigate the effects, safety and tolerability of ularitide as treatment of refractory ascites in cirrhosis patients in a randomised, double-blind, placebo-controlled trial. Participants receive ularitide or placebo as a continuous intravenous infusion during hospitalisation as an add-on to any diuretic treatment. Clinical end points include increase in diuresis and natriuresis, reduction in body weight and waist circumference, safety end points, as well as changes in plasma concentrations of renal and systemic response biomarkers and hormones.

CONCLUSION This study will provide evidence concerning the potential of ularitide in treating cirrhosis patients with refractory ascites.

FUNDING This investigator-initiated trial is supported by ADS AIPHIA Development Services AG.

TRIAL REGISTRATION Clinicaltrials.gov (NCT04311489) and EU Drug Regulating Authorities Clinical Trials (EudraCT: 2019-002268-28). The trial will be conducted in accordance with good clinical practice, the Declaration of Helsinki and applicable demands from Danish authorities.

Ascites is a frequent manifestation of cirrhosis decompensation. Ascites formation heralds a poor long-term prognosis and is associated with discomfort and decreased quality of life [1].

The development of ascites involves numerous patho-mechanisms. Portal hypertension induces vasodilation of the splanchnic vascular system with splanchnic blood pooling. This results in a decreased effective blood volume which causes arterial hypotension. To counteract this systemic hypotension, the organism responds with vasopressin and endothelin secretion, as well as activation of the sympathetic nervous system and the renin-angiotensin-aldosterone-system (RAAS) to facilitate vasoconstriction along with salt and water retention [1, 2].

However, the handling of ascites in cirrhosis with anti-mineralocorticoids, loop-diuretics and a low-sodium diet is generally an effective treatment. Even so, around 10% of patients with ascites become refractory to such

pharmacotherapy and develop refractory ascites [3]. The generally accepted definition of refractory ascites consists of two distinct subgroups; diuretic-resistant, in which ascites does not resolve on maximum dose of diuretics (400 mg spironolactone and 160 mg furosemide daily), and diuretic-intractable due to complications induced by high-dose diuretic treatment [4].

For the past 30 years, the pharmacological treatment strategy for ascites has remained unchanged. No approved drug is available for treatment of refractory ascites, though continuation of diuretics on the highest tolerable dose is justified when renal sodium excretion exceeds 30 mmol/day [4]. At the debut of refractory ascites, patients are facing a gloomy prognosis, and a need for frequent hospitalisation due to complications or for repeated paracenteses [3, 5, 6].

Ularitide is the chemically synthesised form of urodilatin, a human endogenous renal natriuretic peptide synthesised in the distal tubular cells and transported to the inner-medullary collecting duct. Through binding to natriuretic peptide receptors, ularitide suppresses sodium and water reabsorption, producing increased natriuresis and diuresis [7]. This local renal effect of ularitide along with a possible systemic inhibitory effect on RAAS [8] and endothelin [9] may counterbalance patho-mechanisms in refractory ascites.

Ularitide has been tested in two pilot clinical trials in patients with cirrhosis and ascites. The first study administered ularitide intravenously (IV) at a dose of 20 ng/kg/min. over 60 min. to 15 cirrhosis patients with ascites. Ularitide significantly increased the urine flow rate and the urine sodium excretion rate [10]. The second pilot study administered ularitide to seven cirrhosis patients with refractory ascites at a dose of 20 ng/kg/min. for 90 min. The treatment induced a transient significant increase in urine sodium excretion versus baseline, whereas no effect was observed for placebo. Ularitide also increased urine volume, but the effect was not statistically different from that of placebo [11].

In summary, the pharmacodynamics of systemically administered ularitide may be beneficial for cirrhosis patients experiencing pronounced sodium and water retention manifested as refractory ascites.

We hypothesised that a ularitide infusion for a longer-term period (up to 48 h) and at a higher starting dose of 30 ng/kg/min than used in the pilot studies may be effective in inducing and maintaining a clinically meaningful diuresis and natriuresis in patients with cirrhosis and refractory ascites.

METHODS

Patient selection and randomisation

The first patient was enrolled in August 2020. In total, 38 individuals with liver cirrhosis and refractory ascites will be included in this prospective single-centre, double-blind and placebo-controlled randomised clinical trial. Participants are recruited from the Department of Hepatology and Gastroenterology at Aarhus University Hospital (AUH), Denmark. Eligible patients who have provided written informed consent will be screened for in- and exclusion criteria (**Table 1**). Included patients will be randomised to receive treatment with ularitide or a matching placebo in a 2:1 ratio as an add-on to any diuretic treatment. Unequal allocation is chosen to mitigate the likelihood of participant withdrawal and to gain knowledge about safety and efficacy.

TABLE 1 Inclusion and exclusion criteria.

Inclusion criteria
≥ 18 yrs, men and women
Cirrhosis
Refractory ascites[a]
Urine sodium excretion rate < 60 mmol/24 h
Serum creatinine concentration < 150 µmol/l
Child-Turcotte-Pugh-score B7-C13
Bilirubin concentration < 150 µmol/l
Prothrombin time: 0.20-0.60[b]
Systolic blood pressure > 95 mmHg
Written informed consent
Exclusion criteria
Spontaneous bacterial peritonitis and/or gastrointestinal bleeding within 2 weeks prior to inclusion
Proteinuria > 500 mg/24 h
Haemoglobin concentration < 5.5 mmol/l
Hepatic encephalopathy grade 2-4, West Haven classification
Loculated ascites
Primary kidney disease and/or obstructive uropathy
Congestive heart failure
Acute-on-chronic liver failure
Systemic inflammatory response syndrome and acute infections
Known HIV infection
Allergy to natriuretic peptides
Nephrotoxic drugs besides diuretics within the past 1 month
Fertile women not using contraception and/or positive hCG test
Treatment with dobutamine, levosimendan, milrinone, phosphodiesterase inhibitors, octreotide, midodrine, vasopressin, dopamine or other vasopressors within the past 2 weeks

hCG = human chorionic gonadotropin; INR = international normalised ratio.
a) Failure to respond or intolerance to high-dose diuretics: 400 mg spironolactone and 160 mg furosemide daily, resulting in: ascites that cannot be mobilised: ≥ 2 paracentesis within the past 3 months or early ascites recurrence: grade 2-3 ascites within 4 weeks of initial mobilisation.
b) INR: 1.3-2.5.

The randomisation is computer-generated and consists of blocks with four ularitide and two placebo treatments. Treatment randomisation was performed at the packaging facility (Fischer Clinical Services, GmbH, Germany) before shipment to the study centre. Thus, all investigators, study centre personnel and participants will be blinded. Sealed code breakage cards are located at the study centre for use in emergency situations.

Interventions and study design

The ularitide study medication consists of a sterile lyophilised powder and is supplied in a clear glass vial. The formulation comprises 2.5 mg of the active ingredient ularitide and mannitol. Similarly, placebo consisting of mannitol is supplied as a lyophilised powder in a glass vial. Test products are intended for IV use after

reconstitution and dissolution in sterile saline. The final mannitol concentration in the infusion bag is below the concentration of osmotically active mannitol solutions.

Patients will continuously receive ularitide or a placebo infusion via a peripheral IV catheter and remain hospitalised through the 48-h infusion period and the 6-h post-infusion follow-up period; overall 54 h, assuming that no stopping criteria occur. Blood and urine samples are collected as outlined in **Table 2**. Additional blood and urine samples are collected for a biobank for future research.

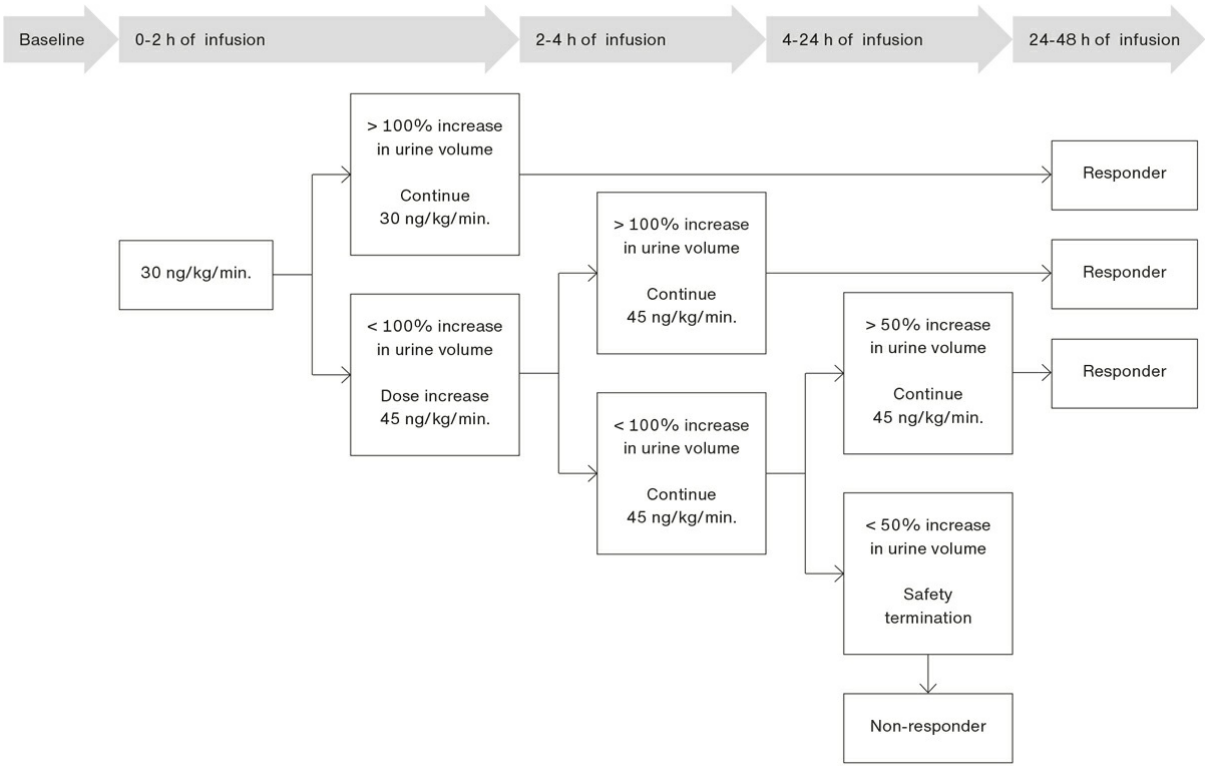
TABLE 2 Pre-treatment, during treatment and post-treatment period assessments.

	OC	Hospitalisation							OC
	inclusion	pre-treatment	during treatment, h[a]				in case of early termination	post-treatment F/U 54 ± 1 h[b]	post-treatment F/U 30 ± 3 days[c]
Assessment	screening	baseline	2 ± ¼	4 ± ¼	24 ± 1	48 ± 1			
Standard samples[d]	x	x			x	x	x	x	
Urine volume	x	x	x	x	x	x	x	x	
Urine sodium excretion rate	x	x	x	x	x	x	x	x	
Urine potassium	x	x	x	x	x	x	x	x	
Urine osmolality		x			x	x	x	x	
Plasma osmolality		x			x	x		x	
Plasma creatinine	x	x	x		x	x	x	x	
eGFR	x	x	x		x	x	x	x	
GFR-24 h-Crea[e]	x				x	x			
Plasma cGMP		x	x		x	x		x	
Plasma copeptin		x	x		x	x		x	
Plasma renin		x	x		x	x		x	
Plasma angiotensin		x	x		x	x		x	
Plasma aldosterone		x	x		x	x		x	
Body weight	x	x			x	x	x		x
Waist circumference	x	x			x	x	x		x
SAE			x	x	x	x	x	x	x

eGFR = estimated glomerular filtration rate; F/U = follow-up; GFR-24 h-Crea = GFR based on 24-h creatinine clearance; GMP = cyclic guanosine monophosphate; INR = international normalised ratio; OC = outpatient clinic; SAE = serious adverse events.
a) Time post infusion start.
b) F/U 6-h post-treatment termination.
c) F/U 30 days after end of treatment.
d) Haemoglobin, red blood cell count, platelet count, total white blood cell count, haematocrit, C-reactive protein, sodium, potassium, urea, bilirubin, creatinine, eGFR, albumin, alanine aminotransferase, alkaline phosphatase, prothrombin time and INR.
e) From the 24-h urine collection at inclusion will be used as baseline.

A graphical overview of how the study is conducted is provided in **Figure 1**.

FIGURE 1 Flow chart of the treatment algorithm.



Before treatment start, baseline urine will be collected over a period of 2 h. All patients must drink the same pre-defined volume of water (2.5 l/24 h) during the treatment and follow-up periods with 200 ml at initiation of baseline and 200 ml before infusion initiation.

This study design addresses the concept of continuous IV infusion of ularitide or placebo at a starting dose of 30 ng/kg/min. Hence, 20 ng/kg/min was the dosage in both ularitide pilot studies and was not as effective in inducing diuresis in individuals with refractory ascites as non-refractory ascites [10, 11]. In the latest pilot study, patients with refractory ascites [11] showed a highly variable diuretic response suggesting that the ularitide dose of 20 ng/kg/min may have been too low to maintain a clinically meaningful diuretic effect. With increasing treatment doses, blood pressure drops may be more pronounced [12]. However, since cirrhotic patients with refractory ascites have varying severities of decompensation and thus varying degrees of sodium and water retention, this trial is designed to individualise treatment doses by up- and down-titration of the infusion dose within a predefined dose range. No significant overall blood pressure drops were reported in the pilot studies.

The dose of 30 ng/kg/min will be maintained for the whole study period if a clinically meaningful effect, ≥ 100% increase in urine volume versus baseline, is observed after 2 h (responders). If no such effect is observed, the dose will be increased to 45 ng/kg/min and maintained for another 2 h. If the increase in urine production at this time point is ≥ 100% versus baseline, treatment continues at the same dose in the remaining study period (responders). If the increase in urine production after 4 h is < 100% versus baseline, treatment continues at the same dose for another 20 h. If patients dosed at 45 ng/kg/min show a ≥ 50% increase in urine volume versus baseline at 24 h post-treatment initiation, they are continuously treated at this dose in the remaining study period (responders). If not, treatment is terminated early for safety reasons (non-responders) (Figure 1). Furthermore, incorporation of a transient treatment stop and an infusion rate reduction to 15 ng/kg/min make it possible to continue treatment of participants who develop hypotension (< 90 mmHg), assuming that these patients are more sensitive to ularitide. Blood pressure will be measured every 15 min for the first 6 h of infusion

and thereafter at least once every 6 h.

The maximum treatment duration is 48 h, which is followed by 6 h post-treatment monitoring. Participants are followed-up at day 30 ± 3 in the outpatient clinic with the primary objective of registering late-onset serious adverse events.

Outcomes

This trial investigates clinical, renal and systemic responses to ularitide in patients with liver cirrhosis and refractory ascites.

Primary efficacy end point:

- Change in urine volume at 24 h after infusion start and at the end of treatment versus baseline.

Main secondary end points:

- Change in the sodium excretion rate at 24 h after infusion start and at the end of treatment versus baseline.

- Change of absolute bodyweight at the end of treatment versus baseline.

Subsequent secondary end points:

- Number of responders per treatment group.

- Absolute and relative changes in diuresis and natriuresis at other time points.

- Changes in plasma and urine osmolality.

- Changes in haematocrit, serum creatinine as well as plasma concentrations of cyclic guanosine monophosphate (cGMP), copeptin, renin, angiotensin and aldosterone.

- Changes in bodyweight and waist circumference are considered surrogate measures of the ascites burden, and reductions from baseline are measured after 24 and 48 h of infusion and at the 30-days post-treatment follow-up.

- Relevant safety precautions are incorporated in the study design and treatment will be discontinued prematurely if a predefined stopping criterion presents; thus,

- Safety outcomes comprise incidence of adverse events, serious adverse events and stopping criteria leading to a dose reduction or early termination of treatment in the whole study period.

Sample size calculation

For sample size estimation, data from the first and larger pilot study [10], with a two-to-three-week wash-out period between treatment sequences and similar baseline values of urine flow rate, were used. The assumed mean increase in urine flow rate is 2.0 for the ularitide group and –1.0 for the placebo group. Group sample sizes of 25 ularitide treatments and 13 placebo treatments achieve 80.8% power to reject the null hypothesis of equal means when the difference between population means is 3.0 with standard deviations (SDs) of 3.8 for the ularitide group and 2.5 for the placebo group, and applying a significance level of 0.05 using Satterthwaite's two-sided two-sample unequal-variance t-test.

Data management and data analysis plan

Data collection will be conducted by the investigators as well as entered and managed using REDCap electronic data tools hosted by Aarhus University, Denmark [13, 14]. The final dataset will be owned by the Department of Hepatology and Gastroenterology, AUH. The GCP units at Aarhus and Aalborg University Hospitals are monitoring the trial. Handling and reporting of adverse events and serious adverse events comply with

applicable guidelines and requirements for clinical trials in Denmark and the EU.

The primary end point will be evaluated separately for responders and non-responders. The χ^2 test will be used for categorical values. Paired statistics will be used for investigation of pre- versus post-treatment effects. Data will be presented as mean ± SD for normally distributed data and median/range for non-normally distributed data.

Trial registration: Clinicaltrials.gov (NCT04311489) and EU Drug Regulating Authorities Clinical Trials (EudraCT: 2019-002268-28). The trial will be conducted in accordance with good clinical practice, the Declaration of Helsinki and applicable demands from Danish authorities.

DISCUSSION

Participants and safety precautions

Patients with refractory ascites have restricted treatment options and the majority receive frequent paracentesis to relieve the discomfort of ascites. Hence, this study intends to investigate a new drug for the treatment of refractory ascites, potentially boosting the pallet of treatment options. The current formulation of ularitide requires IV infusion. Ularitide has a half-life of a few minutes; and by using IV administration, the treatment is easily adjusted or terminated by the investigators, ultimately increasing safety for the participants. If the present study demonstrates meaningful effects of ularitide, alternative administration ways warrant consideration, e.g. subcutaneous infusion or pills.

Ularitide has been extensively tested in clinical studies for varying indications such as heart failure, acute renal failure and asthma. Its safety profile is well known with hypotension being the most frequently reported adverse reaction. Other common adverse reactions are dizziness, headache, hyperhidrosis, hypokalemia and nausea. Safety precautions and close monitoring of participants during hospitalisation are integrated in this clinical trial set-up. Furthermore, the study is designed with a crossroad after 24 h of treatment, where non-responders will be terminated. This design rests on ethical considerations. Included patients have a sodium excretion rate below 60 mmol per 24 h. Investigational medicines must be dissolved in isotonic sodium chloride; therefore, placebo-treated individuals will receive a load of sodium but with limited excretion capacity, ultimately aggravating their ascites. Thus, an increase in urine output must be present, with 24 h as the latest time point before further treatment is justified. Oppositely, responders will likely experience a beneficial effect of the treatment through relief of ascites-related discomfort.

Participation is in accordance with the General Data Protection Regulation and the Danish Data Protection Act.

Dissemination

Results will be published and presented at national and international conferences. We will establish a biobank for future research, providing an opportunity to perform relevant future analysis for the benefit of cirrhosis patients.

The International Committee of Medical Journal Editors' recommendations apply when authorships are credited [15]. We used the SPIRIT checklist when drafting our report [16].

CONCLUSION

This project aims to elucidate a new possible pharmacological treatment of patients with liver cirrhosis and refractory ascites and to potentially significantly alter the existing treatment algorithm. Long-term ularitide

infusion has never been investigated with this indication. The present trial will include more participants with liver cirrhosis and refractory ascites than have ever before been exposed to ularitide. Hence, this study may determine the diuretic and natriuretic potential of ularitide in treating cirrhosis patients with refractory ascites. In case the current proof-of-concept study demonstrates promising results, larger and longer lasting phase II/III trials are necessary to investigate the clinical implications of add-on ularitide treatment on, e.g., paracentesis interval, portal pressure and survival.

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Conflicts of interest Potential conflicts of interest have been declared. Disclosure forms provided by the authors are available with the article at ugeskriftet.dk/dmj

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