Inhaled nebulised unfractionated heparin (UFH) for the treatment of hospitalised patients with COVID-19: A randomised controlled pilot study.

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November 8, 2022

Abstract

There is a strong scientific rationale to use nebulised unfractionated heparin (UFH) in COVID-19. This pilot study investigated whether nebulised UFH was safe and had any impact on mortality, length of hospitalisation and clinical progression, in the treatment of hospitalised patients with COVID-19. This parallel group, open label, randomised trial included adult patients with confirmed SARS-CoV-2 infection admitted hospital in Brazil. One hundred patients were planned to be randomised to either "standard of care" (SOC) or SOC plus nebulized UFH. The trial was stopped after randomisation of 75 patients due to falling COVID-19 hospitalisation rates. Significance tests were 1-sided test (10% significance level). The key analysis populations were intention to treat (ITT) and modified ITT (mITT) which excluded (from both arms) subjects admitted to ITU or who died within 24 hrs of randomisation. In the ITT population (n=75), mortality was numerically lower for nebulised UFH (6 out of 38 patients; 15.8%) versus SOC (10 out of 37 patients; 27.0%), but not statistically significant; odds ratio (OR) 0.51, p=0.24. In the mITT population, nebulised UFH reduced mortality (OR 0.2, p=0.035).

Introduction

Since December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected millions of people globally, with many patients requiring hospitalisation and ventilatory support. The pathophysiology of COVID-19 is characterised by microvascular thrombosis and coagulopathy accompanied by diffuse alveolar damage, inflammation, DNA neutrophil extracellular traps (NETS) and hyaline membrane formation [1]. We previously described the scientific rationale for nebulised unfractionated heparin (UFH) to

be a potentially effective treatment for COVID-19 because of its broad anti-inflammatory activity combined with its anti-coagulant and anti-viral effects [1].

In early-phase trials in patients with acute lung injury, nebulised UFH reduced microvascular thrombosis and hypercoagulation, improved pulmonary dead space and reduced lung injury, with increased time free of ventilatory support [2-4]. Furthermore, in a pre-pandemic, multicentre, double-blind, randomised controlled study in 256 critically ill ventilated patients, nebulised UFH limited survivors' lung injury and accelerated their return to home [5]. Nebulised heparin has also been shown to benefit patients with a number of other respiratory conditions including ARDS [6] and COPD [7], importantly without major safety concerns.

We and others have shown that heparin is able to bind the spike protein expressed by the SARs-CoV-2 virus [8, 9] and that UFH in particular reduced the ability of the virus to infect cells in vitro. SARS-CoV-2 Spike protein binding to human epithelial cells requires the engagement of both cell surface heparan sulphate (HS) and angiotensin-converting enzyme 2 (ACE-2), with HS acting as a co-receptor for ACE-2 interaction. UFH has the ability to compete for binding of the SARS-CoV-2 S protein to cell surface HS, and thus reduce infectivity [8]. Additionally, the SARS-CoV-2 Spike S1 protein receptor-binding domain attaches to UFH and undergoes a conformational change that blocks the binding to the AC2 receptor and infectivity of SARS-CoV-2 to Vero E6 cells [9]. This inhibition of SARS-CoV-2 infection of Vero E6 cells by UFH is concentration-dependent, occurs at therapeutically relevant concentrations likely to be achieved following inhalation, and exhibited a significantly stronger anti-viral effect compared to low molecular weight heparins (LMWHs) [8, 9].

These observations suggest that UFH uniquely possesses anti-coagulant, anti-inflammatory, and anti-viral activity [1, 10, 11] of relevance to the treatment of COVID-19. It is now well recognised that the use of systemic heparin provides an overall beneficial effect in the treatment of patients with COVID-19 when administered as part of the standard of care [12]. The rationale for investigating nebulized UFH is that when administered by this route, the drug is retained within the lung and therefore could provide local anti-inflammatory, anticoagulant, and antiviral activity on top of the effect of systemic heparin and other treatments recognised as standard of care; thus, local availability of heparin in the lung following nebulisation may ensure sufficient airway luminal concentrations capable of reducing viral infection, alveolar coagulation and inflammation above that achieved with systemic administration of heparin.

If efficacy is confirmed, the low cost of the drug may make this treatment accessible for low- and middle-income countries.

Here, we report the safety and efficacy of nebulised UFH in a pilot study in patients with COVID-19 admitted to two hospitals in the state of Sao Paulo, Brazil during the regional peak of the pandemic with the delta variant of SARs-CoV-2. This trial also forms part of a larger meta-trial established to investigate the potential benefit of nebulised UFH in hospitalised COVID-19 patients [13]

Methods

This pilot trial was an open, randomised, parallel group study investigating the effect of nebulised unfractionated sodium heparin (Cristalia Ltda; Brazil, 25,000 IU) in patients admitted to two hospitals in the state of Sao Paulo, Brazil (Sao Roque Hospital and Santa Casa de Sorocaba Hospital) between February 25th, 2021, and July 14th, 2021. The trial identification UTN code is U111-1263-3136 and all patients gave their written informed consent to participate in this study which was approved by the Ethics Committee of the University of Sao Paulo – Biomedical Sciences Institute.

Study treatment

Nebulised UFH was administered through a vibrating mesh nebuliser (NebZmart, Glenmark, Taiwan) with 1.25 ml of the sodium heparin (5,000 IU / 0.25 mL) being diluted with 4 ml of 0.9% saline at 6 hourly intervals in addition to standard of care, with other patients randomised to receive only standard of care as the control arm of the study. Randomisation was 1:1 without any adjustment for any clinical characteristics. Randomisation was produced by using a computerized random list generator.

The UFH used was formulated as ampules containing 5,000 IU/0.25 mL, from Cristalia Ltda, meeting all the requirements of the USP and Brazilian Pharmacopeia (BP). The heparin samples were analyzed by 1D^{1}H-NMR spectroscopy, gel permeation and anion exchange chromatographies and had their anticoagulant potencies determined with anti-FIIa and anti-FXa activity assays, as required by both the USP and BP. The molecular mass distribution parameters of the formulations also met the requirement of USP. Besides certified as being from porcine origin, the formulations showed no contamination with heparins from other animal sources, as recommended by the BP. The 1D^{1}H-NMR spectrum show no signal at 7.4 ppm, assuring the absence of benzyl alcohol commonly added as a preservative in multiuse vials for intravenous administration but not into formulations intended for subcutaneous use. Furthermore, the formulations used showed no contamination with oversulfated chondroitin sulfate after analyses by NMR and anion exchange chromatography.

Study population

Patients with a positive SARS-CoV 2 RT PCR test who required hospitalisation were included in the trial. Patients were required to have a WHO modified ordinal clinical scale (MOCS) score (Table 1) of 3 – 5; subjects requiring mechanical ventilation were not eligible. Exclusion criteria included known hypersensitivity to heparin or related compounds, or the diagnosis of a pulmonary obstructive disease. Any patients requiring admission to ICU ceased to receive any further treatment with nebulised UFH.

We collected the following baseline demographic and clinical data: age, sex, co-morbidities, WHO modified ordinal clinical scale (MOCS) for COVID-19, as shown in Table 1, level of respiratory support, other treatments reflecting the standard of care in use during the time of the trial, including the use of any prophylactic or therapeutic anti-coagulant treatment. We also collected the following treatment-related variables: nebulised UFH dose (daily and cumulative), treatment duration, missed doses and reasons for this. The following safety outcomes were also assessed: baseline and peak activated partial thromboplastin time (APTT), the incidence of epistaxis, blood-stained sputum, pulmonary bleeding, major bleeding, heparin-induced thrombocytopenia, and any other serious adverse events.

Table 1. WHO modified ordinal clinical scale (MOCS) for COVID-19

Level	Description
	Not hospitalised
	Hospitalised, not requiring supplemental oxygen and no longer requiring medical care for COVID-19
	Hospitalised not requiring supplemental oxygen, but in needing medical care for COVID-19
	Hospitalised requiring supplemental oxygen
	Hospitalised requiring non-invasive ventilation or high-flow oxygen
	Hospitalised requiring intubation and mechanical ventilation or ECMO
	Death

Outcomes

Two primary outcomes were measured in this pilot study: the mortality rate and the length of stay in hospital (in days) in each arm. The secondary outcomes were: Modification of the WHO ordinal scale (clinical parameters) on every day but analysis was performed only at days 1 (baseline), 7, 15 and 29 (or until the end of hospitalisation), oxygen-free survival days and mechanical ventilation rates.

Statistical Analysis

Since this was a pilot study, no sample size or power calculations were used; We aimed to recruit 100 patients

as a pragmatic approach to evaluate efficacy signals that could promote larger studies of nebulised UFH for the treatment of hospitalised patients with COVID-19. As this pilot study was not powered for definitive efficacy analysis, significance tests were 1-sided with a 10% significance level, an approach used in early phase clinical trials[14].

Treatment effects on survival rate were analysed by Fisher‘s exact text, while length of stay was analysed by Wilcoxon signed ranked test. In case of death during the study, a value of 29 days was assigned for the number of days of hospitalization. The time to hospital discharge was estimated by use of the Kaplan-Meier method and Hazard Ratio risk estimates by Cox model proportional risk analysis. The change in MOCS was analysed by Mann-Whitney. The number of days that each subject was both oxygen free and alive up to day 29 was determined. Fisher‘s exact text was used to assess the effect of UFH on the requirement for mechanical ventilation.

Both primary and secondary outcomes were measured using 3 datasets, with the ITT and mITT being the primary populations of interest:

ITT (Intention-to-treat), corresponding to all subjects who were randomised and, for those in the active treatment group, received at least one dose of nebulised UFH, and participated in at least one post-baseline assessment.

A modified ITT (mITT) population which excluded (from both arms of the ITT population) subjects admitted to ITU within 24 hrs of randomisation and subjects who died within 24 hrs of randomisation. Patients randomised to active treatment were required to have received at least 4 administrations of nebulised UFH, corresponding to 24 hours after randomisation.

PP (Per protocol), corresponding to all subjects who adhered to the major criteria in the protocol (e.g. all subjects who completed efficacy analyses, whose study drug compliance was between 75% and 125%), and all subjects who did not substantially deviate from the protocol. In addition, a post hoc analysis of the sub-group of PP patients who received at least 6 administrations of UFH was analysed; this group was called PP6.

Safety outcomes

Baseline activated partial thromboplastin time (APTT) levels before, and on days 3 and 5 following initiation of treatment with nebulised UFH, were measured, as well as recording of any adverse events in all randomized subjects.

Results

Seventy-six patients were recruited when admitted to the hospital. Seventy-five patients were randomised, according to Figure 1. Consort statement and patient allocation.

Table 2 . Baseline characteristics and additional characteristics

	All patients (n=75)	**Standard of care** (n=37)	**Heparin** (n=38)	**P-value**
Age (y)	51.95 ± 12.39	52.63 ± 12.48	51.26 ± 12.27	0.688
Male (n,%)	48 (63%)	25	23	N/A
Caucasian	65 (85%)	35	30	N/A
Body Mass Index (kg/m2)	30.65 ± 6.19	29.79 ± 6.02	31.44 ± 6.23	0.3897
Time from symptom onset (days)	8.61 ± 4.15	8.97 ± 5.10	8.26 ± 2.85	0.9329

	All patients (n=75)	**Standard of care** (n=37)	**Heparin** (n=38)	**P-value**
Time to commencement of inhaled UFH (hours)	N/A	N/A	4	N/A
Modified ordinal scale at baseline (average)	4.23 ±0.57	4.14 ± 0.58	4.32 ± 0.61	0.2054
WHO SCORE 1 2 3 4 5 6 7	Number of patients 0 0 8 44 24 0 0	Number of patients 0 0 4 24 9 0 0	Number of patients 0 0 4 20 15 0 0	Number of patients N/A

N/A=not applicable

The baseline characteristics of the patients are summarised in Table 2. Patients were on average 51.5 years old and 63.2% were males. The majority of patients enrolled in the trial were Caucasian. No significant differences were observed between groups at baseline for age or body mass index. There were also no significant differences observed between groups at baseline for time from symptom onset, time from hospital admission and with the WHO modified ordinal scale at admission.

The time to commencement of inhaled nebulised UFH ranged from a minimum of 1 hour to a maximum of 27 hours following hospital admission, with a median time of 4 hours. Most patients at the two hospitals received a range of other treatments as standard of care, including corticosteroids (dexamethasone), antibiotics (ceftriaxone and azithromycin) and anticoagulants (typically, enoxaparin sodium intravenous (IV) or subcutaneous (SC), either in prophylactic or therapeutic doses). The standard of care treatment was not different between the two arms of the trial and included

The trial was stopped when no patients had been hospitalised with COVID-19 for 60 consecutive days in either of the two sites engaged in this trial.

Mortality

In the ITT population (n=75), mortality was numerically lower for nebulised UFH on top of standard of care (6 out of 38 patients; 15.8%) versus standard of care (10 out of 37 patients; 27.0%), but this difference was not statistically significant (odds ratio 0.51, p=0.2349), with similar results observed for the per protocol population (odds ratio 0.31, p=0.1482) (Table 4). However, there were statistical differences between treatments in favour of nebulised UFH in the mITT population (odds ratio 0.2, p=0.0353), and the post-hoc analysis of PP6 population (odds ratio 0.1, p=0.0184), Table 4.

All deaths occurred in patients who scored 4 or 5 on the WHO ordinal scale on the day of hospital admission.

Table 3 -Mortality comparison between the two groups)

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Days of hospitalisation

Subjects receiving nebulised UFH showed a similar time to discharge compared to the standard of care group.

Table 4 – Days of hospitalization comparison

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Mann-Whitney, P-values based on median.

Oxygen-free survival days

Subjects receiving nebulised UFH had a similar number of oxygen free survival days compared to the standard of care group (Table 6).

Table 5 – Days of oxygen use in all datasets

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Mann-Whitney, P-values based on median.

WHO MOC

There was a significant treatment benefit for patients receiving nebulised UFH on top of standard of care versus patients treated with standard of care only as assessed by the WHO MOCS (Table 7). At day 29, the treatment difference reached significance in all analysis populations ($p<0.1$).

Table 6 – Evolution of the MOOC WHO scale following treatment with standard of care or heparin plus standard of care

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Mechanical ventilation rates

The rates of mechanical ventilation were not significantly different between the two treatment arms in the ITT population, although the nebulised UFH treatment arm showed numerically lower rates of intubation (ITT population; odds ratio 0.51, 95% CI 0.16 – 1.57)(Table 8). For the MITT and PP populations, the treatment effects reached the pre-defined level of significance ($p<0.1$).

Table 7 – Rates of Mechanical ventilation

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Fisher exact test

Safety Outcomes

No laboratory measurements showed abnormal findings that could be attributable to treatment with nebulised UFH and in particular, there were no significant differences in APTT between the two treatment groups.

There were no cases recorded of pulmonary bleeding, heparin induced thrombocytopaenia, or anaphylaxis during or immediately after administration of nebulised UFH, or any other adverse events.

Discussion

This exploratory study showed clinical benefit for nebulised UFH on top of standard of care for patients hospitalised with COVID-19. The primary endpoints showed a reduction in mortality, reaching significance in the mITT population, but no difference in duration of hospital stay. For the secondary endpoints, positive effects of nebulised UFH were observed on MOCS change and rates of mechanical ventilation. Overall, nebulised UFH appeared to be well tolerated.

The two analysis populations of key interest were the ITT and mITT. The purpose of the mITT was to exclude subjects from both treatment arms with rapid disease progression within 24 hours of admission, resulting in mechanical ventilation or death. There is limited scope for nebulised UFH to alter the disease trajectory in these subjects, as their disease trajectory implies a poor prognosis. Furthermore, these subjects received few (<4) or no UFH doses before ventilation or death; the potential benefits of nebulised UFH are unlikely to be realised after such short treatment duration is subjects with rapidly progressive disease. While the mortality benefit in this study was greatest (and significant) in the mITT population (odds ratio 0.20), a numerical (non-significant) trend was observed in the ITT population (odds ratio 0.51). A post-hoc analysis of subjects who received at least 6 nebulised UFH doses showed a greater benefit (odds ratio 0.1, p=0.0184), compatible with the concept that at least 24 hrs UFH treatment allows a greater possibility for the clinical benefit of this treatment to be realised.

The secondary endpoints for MOCS change and rates of ventilation showed evidence for nebulised UFH clinical efficacy. Taken together with the mortality efficacy signals observed, these results support larger studies of the benefits of nebulised UFH on COVID-19. Our results will also be used as part of an ongoing meta-trial involving a higher number of patients with COVID-19 [1]. The results of this study are consistent with a recently published case series of 98 patients showing that nebulised UFH administration in hospitalised patients with COVID-19 is safe and potentially beneficial [15], and earlier studies reporting a beneficial effect in patients with ARDS pre-pandemic [5].

The duration of hospital stay (co-primary endpoint) did not show any differences between groups. This outcome measure can be influenced by a variety of factors including age, co-morbidities and rate of recovery from COVID-19. It is probable that our study was too small to see any difference in this outcome measure. Additionally, this endpoint was likely influenced by hospital pressures during the peak of the pandemic to create space for newly diagnosed COVID-19 patients by the rapid (subjective) discharge of improving patients.

We had planned to recruit a sample size for this trial of 100 study subjects. However, we only managed to recruit 76 patients into the study due to changes in the prevalence of COVID-19 patients in the state of Sao Paulo between February 25th, 2021, when the study started and July 14th, 2021 when the Steering Committee decided to end recruitment and analyse the data. The smaller than anticipated study size the possibility to conduct sub-group analysis. Other limitations included the heterogeneity of the clinical status of patients on admission to hospital, the change in vaccination status through the progress of the trial and the open label status of the treatment arms.

Whilst our study is in a limited number of patients, it suggests that use of nebulised UFH is safe and may provide additional benefit in reducing mortality in patients hospitalised with COVID-19 on top of standard of care, including patients who were also receiving systemic heparin [12, 16]. Whilst a number of effective treatments have now been identified to treat hospitalised patients with COVID-19 [15], the recent emergence of new variants of SARs-CoV-2, that appear to be highly transmissible and possibly escape the impact of many of the available vaccines, shows that there is still a need to continue to seek effective treatments for this and future virally mediated pneumonias. The ability of heparin administered by inhalation to be anti-viral and prevent infection of mammalian cells with SARs-CoV [1, 11], coupled with its well-recognised ability

to reduce inflammation by binding various cytokines implicated in the cytokine storm often associated with COVID-19 [1, 10], as well as the well-recognised anti-coagulant effect of this drug of benefit in dealing with the alveolar coagulation seen in such patients, suggests that further controlled, larger studies of nebulised UFH are warranted, given that this a widely available and affordable medicine.

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