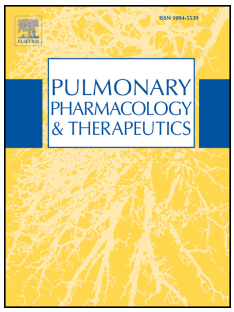

Inhaled nebulised unfractionated heparin improves lung function in moderate to very severe COPD: A pilot study

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Inhaled nebulised unfractionated heparin improves lung function in moderate to very severe COPD: A pilot study

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Abstract

Background; COPD is an inflammatory airway disease characterised by progressive airflow limitation and air trapping, leading to lung hyperinflation and exercise limitation. Acute worsening of symptoms, including dyspnea, cough and sputum production, occurs during exacerbations which are associated with significantly reduced health related quality of life, and increased morbidity and mortality. Chronic bronchial mucus production and productive cough are risk factors for exacerbations. Medicines targeting bronchoconstriction and airway inflammation are the current mainstays of COPD therapy. However, there is growing concern with an increased risk of pneumonia in patients with COPD receiving regular inhaled corticosteroids and there is therefore a need to find safer alternative treatments.

Previous studies have indicated that inhalation of unfractionated heparin (UFH) treats local inflammation, mucus hypersecretion and lung injury, without systemic anticoagulation, and is safe. Therefore, our primary objective was to demonstrate that inhaled UFH significantly improves lung function (FEV_1) over 21 days of treatment in patients with COPD receiving pulmonary rehabilitation and that UFH provides a novel, safe and effective way of treating this complex disease.

Methods; Forty patients with moderate to very severe COPD admitted to the IRCCS San Raffaele Pisana Hospital for 21 days pulmonary rehabilitation were randomised to receive nebulised inhaled UFH (75,000 or 150,000 IU BID) or placebo for 21 days. All patients also received nebulised salbutamol (1 mg) and beclomethasone dipropionate (400 µg) BID over the same period.

Lung function was measured at day 0, 7, 14 and 21 of treatment and at a follow-up visit 7 days post-treatment. Exercise capacity (6MWT) and dyspnoea (Borg score) were measured before and after treatment.

In pre-clinical studies, the ability of basic proteins found in COPD sputum to neutralise the anticoagulant activity of heparin was determined using the AMAX heparin assay kit.

Main Results; At both doses, UFH significantly increased FVC following 7 days of treatment and 150,000 IU BID significantly increased FEV1 (+249±69 ml compared with placebo) at this time, an effect maintained to

the 28 day follow-up. Clinically significant improvement in exercise capacity and dyspnoea were seen after 21 days of treatment with both doses of UFH. There were no serious adverse events or effects on systemic coagulation. Pre-clinical studies demonstrated that the basic proteins lactoferrin, platelet factor-4 (PF-4), IL-8 and polyarginine, as a model of the eosinophil cationic protein (ECP), found in COPD sputum neutralise the anticoagulant activity of heparin.

Conclusion; Inhaled nebulised UFH is safe and provides additional clinical benefit for patients with moderate to very severe COPD through effects that are independent of its anticoagulant activity.

Key words; COPD, clinical trial, inhaled, unfractionated heparin, lung function, anticoagulant activity.

Introduction

Chronic obstructive pulmonary disease (COPD) is a rising major global healthcare problem associated with significant morbidity and mortality [1]. Current treatment options are limited [2] and none conclusively modify the long-term decline in lung function [3]. Currently available drugs for COPD are mostly bronchodilators [4,5] that provide symptomatic treatment through relief of reversible airflow obstruction, an important, though not universal, feature of COPD [3]. Improvement in airflow obstruction can also result from reduction in airway inflammation or improved clearance of mucus. Current recommendations advocate the use of inhaled long-acting bronchodilators combined with inhaled corticosteroids (ICS) for maintenance treatment of moderate-to-severe COPD [3]. However, patients with COPD are often poorly responsive to corticosteroids and there may be an increased risk of pneumonia in patients taking this class of anti-inflammatory drugs [6]. Recently the orally active PDE4 inhibitor roflumilast has been introduced as an anti-inflammatory drug for the treatment of COPD, but this has been restricted to use on top of existing therapies in severe patients, and is dose-limited by adverse effects, particularly in the gastrointestinal system [7]. There is therefore a need to find new therapeutic approaches for the treatment of COPD and a number of novel bronchodilators [8], anti-inflammatory drugs [9] and bifunctional agents [10] are under development for the treatment of this disease.

Studies with older mucolytic/anti-oxidant drugs such as N-acetylcysteine [11], carbocysteine [12] and more recently erdosteine [13] have provided evidence that targeting these components of the disease has clinical benefit in reducing exacerbations in patients with COPD. We have therefore investigated the effect of inhaled nebulised unfractionated heparin (UFH) as this drug exhibits many pharmacological properties including mucolytic, anti-inflammatory, anti-oxidant and wound healing properties relevant to the treatment of COPD [14,15].

Heparin belongs to the family of polyanionic glycosaminoglycans (GAGs), polysaccharides composed of hexuronic acid and D-glucosamine residues joined by glycosidic linkages [16]. We previously showed that when subcutaneous low molecular weight heparin (enoxaparin), 20 mg daily, was added to inhaled

salmeterol/fluticasone propionate in patients with stable moderate COPD, FEV_1 was significantly increased after 4 weeks of additional enoxaparin therapy, compared to a significant improvement after 12 weeks of standard therapy [17]. However, long-term parenteral administration of an anticoagulant is undesirable and many of the beneficial effects of heparin in COPD are likely to be via its non-anticoagulant actions [14]. Inhaled nebulised UFH has previously shown to be safe and without side effects in healthy volunteers [18,19] and in patients with asthma, cystic fibrosis, acute lung injury or idiopathic pulmonary fibrosis, at doses up to 400,000 units [19-23]. The inhaled route for administration of systemic heparin, 20,000 units, was previously investigated in patients with COPD [24], and all of these early trials found minimal absorption of heparin from the respiratory tract and no adverse effects.

Previous studies have indicated that inhalation of UFH treats local inflammation, mucus hypersecretion and lung injury without systemic anticoagulation or any incidence of pulmonary haemorrhage (20 studies, 536 patients) [25], and was safe and effective in patients with smoke inhalation injury, acute lung injury, asthma and allergy, and cystic fibrosis [26]. Heparin has multiple protein binding partners [14, 16], including the basic chemokine platelet factor-4 (PF-4) which is known to neutralise the anticoagulant activity of heparin [27]. We therefore tested the ability of PF-4 and a number of basic proteins, lactoferrin, IL-8 and polyarginine as a model of the eosinophil cationic protein (ECP), found in the normal and inflamed COPD airways, to inhibit the anticoagulant activity of heparin.

Furthermore, in a pilot, randomised, double blind, placebo-controlled clinical study, we have investigated the effect of nebulised UFH on a range of clinically relevant endpoints in patients with moderate to very severe COPD undergoing pulmonary rehabilitation.

Methods

Preclinical studies

The anticoagulant activity of heparin was measured in the presence of excess Factor Xa and anti-thrombin III using the AMAX heparin assay kit (Trinity Biotech, Bray, Ireland). In this assay, the residual Factor Xa is inversely proportional to the heparin concentration. Using this assay in the microtitre plate format, UFH (pig intestinal mucosa, Calbiochem) at 0.5 µg/ml gave nearly complete inhibition of Factor Xa activity (Figure 1). Basic proteins were added to the incubations in the concentration ranges shown to achieve complete neutralisation of the anti-factor Xa activity of UFH. Data was analysed by one-way ANOVA and Dunnetts multiple comparisons test using Graph Pad Prism (version 5) software.

Clinical study design

This study was designed as a 3 week, single-centre, randomised, double-blind, parallel-group, placebo-controlled, clinical trial of nebulised UFH at two doses. The study (EudraCT Number 2010-024168-16) was approved by the local Ethics Committee Board (Registro Pareri E/33/14), and carried out in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from all patients who participated in the trial.

Patients

Patients were recruited from the Pulmonary Rehabilitation ward of the IRCCS San Raffaele Pisana Hospital (Rome, Italy) following admission for treatment, usually within 7 days post-exacerbation. This study enrolled COPD patients (men and women, aged 50 – 85 years) with moderate to very severe airflow limitation (stage II to IV according to Global Initiative for Chronic Obstructive Lung Disease [GOLD] 2014 spirometric classification [3], forced expiratory volume in 1 second [FEV_1] 20 - 70% of normal predicted and FEV_1 / forced vital capacity [FVC]<0.7). Patients had to be current or ex-smokers with a smoking history of at least 20 pack years, (see Table 1).

Exclusion criteria including hemoptysis, bleeding and pre-existing presence of heparin induced antibodies are described in the on-line data supplement.

Randomisation and masking

Forty COPD patients were randomly assigned in a 1:1:1 ratio to receive inhaled nebulized UFH (75,000 IU or 150,000 IU) or placebo using a computer generated permutation, with a block of three subjects.

Treatments

Patients received twice daily treatment with inhaled UFH (75,000 IU or 150,000 IU, molecular weight 13,000, Teofarma, Italy) or placebo (distilled water) for 21 days, nebulised from a jet nebuliser (compressor: SpeedyMed, Italy; nebulizer: Ampolla Poly, Italy), the particles having a mass median aerodynamic diameter (MMAD) of 3.25 µm, and a fill volume of 7 ml.

Dosing was based on our previous unpublished study of inhaled UFH in COPD (n=8, FEV_1%; 52.6 (SD 30.9), in which we found 50,000 IU BID for 14 days was safe, but without effect on FEV_1 and the observation that in healthy subjects 150,000 IU may be the top nebuliser fill before systemic effects are detected [19].

All patients received concomitant medication with salbutamol (1 mg twice daily via nebulisation) and beclomethasone dipropionate (400 µg twice daily via nebulisation), as well as pulmonary rehabilitation. However, in view of the potentially dehydrating effect of anti-cholinergics on airway secretions making mucus more difficult to expectorate [28], long-acting muscarinic receptor antagonists were discontinued with a washout of 7 days before randomization.

The pulmonary rehabilitation programme was as we previously described [29], carried out over a total of 3 weeks.

Objectives, assessments, and outcome measures

The primary objective was to demonstrate a relatively small, but clinically significant, improvement in trough FEV_1 of 140 ml, according to the recommendations of the ERS/ATS taskforce on outcomes for COPD pharmacological trials [30], following inhaled UFH therapy for 21-days compared with placebo.

Secondary objectives were to demonstrate improvements in exercise capacity evaluated as the distance covered in 6 min (6MWD) and dyspnoea (Borg score), with no significant change in blood coagulation parameters, compared with placebo. Further secondary objectives were the influence of UFH on post-exercise pulse oximeter saturation (SpO_2) and heart rate.

At day 1 spirometry was performed before the treatment, and 1 hour and 4 hours post treatment, and blood samples were taken. On day 1 and 21, the 6MWD and Borg questionnaire were assessed. Patients continued with inhaled therapy every 12 hours for 21 days, with weekly coagulation and spirometry testing. The same spirometry and coagulation tests were performed at the follow-up visit one week after the end of the treatment period.

Statistical analysis

Values were reported as means and standard error of the mean (SEM). Two-way analysis of variance (ANOVA) with Bonferroni post-test and t-test were used for treatment and point-by-point comparisons, respectively, for the treated patients. The statistical significance was defined as $P<0.05$, and all data analyses were performed by using the computer software GraphPad Prism version 5.00 for Mac (CA, USA).

Results

In our pre-clinical study, protamine sulphate in 1:1 and 2:1 w:w ratios, those used clinically to neutralise heparin overdose, neutralised the inhibitory effect of heparin (0.5 µg/ml) on Factor Xa activity (Figure 1A), validating the assay we used. We initially confirmed the effect of PF-4 on heparin activity. It has been known for a long time that PF-4 neutralises the anticoagulant activity of heparin and it was first

demonstrated that 10 µg of purified PF-4 neutralised 1 µg of heparin [27]. In our assay we show that PF-4 completely neutralised the anticoagulant activity of heparin in the same ratio (Figure 1B).

Further, we tested the effect of basic peptides and proteins, lactoferrin, IL-8 and polyarginine as a model for arginine rich ECP, found in abundance in the inflamed COPD airway. Lactoferrin at 10 µg/ml completely neutralised the anticoagulant activity of heparin (Figure 1C). On a molar basis lactoferrin is as potent as protamine sulphate in this effect. Polyarginine (molecular weight 15,000 – 70,000), when added at 5 µg/ml completely neutralised the anticoagulant activity of heparin (Figure 1D). IL-8, another basic chemokine, was not as effective as PF-4 in neutralising heparin anticoagulant activity and only at concentrations higher than 50 µg/ml was IL-8 able to neutralise the activity of heparin. Complete inhibition was seen at 90 µg/ml (Figure 1E).

In our clinical study, a total of 40 patients were randomised to receive treatments. Overall, 60.0% of patients completed the study (75% placebo; 46.2% heparin 75,000 IU and 60% heparin 150,000 IU). The majority of treatment discontinuations (56.3%) were related to a high degree of poor compliance with the time for nebulisation (~30 minutes) amongst recruited patients, but were not related to drug treatment (Figure 2). Table 1 shows the baseline characteristics of COPD patients enrolled in the study from February 2012 to March 2015. In the group of patients treated with heparin at the higher dose, by chance, the FEV_1 value expressed as % predicted was significantly lower ($P<0.05$) compared to the placebo group at the start of the study, although this difference was not statistically significant when FEV_1 was expressed as L ($P>0.05$ vs. placebo).

Impact of inhaled heparin on FEV_1 and FVC

Inhaled heparin (150,000 IU) significantly enhanced change from baseline of trough FEV_1 after 7 days of treatment compared with placebo treated patients (+249±69 ml, $P<0.05$) and baseline (+144±69 ml, $P<0.05$) (Figure 3A). Heparin administered at the higher dose also significantly ($P<0.05$) increased the change from baseline of trough FEV_1 during the study period, compared with placebo treated patients, and

this effect was sustained and even greater at the follow-up visit (+99±131 ml vs. day 21, P>0.05) (Figure 3A and B).

Inhaled heparin administered at both low and high doses significantly enhanced the change from baseline of trough FVC after 7 days of treatment compared with placebo treated patients (overall +441±163 ml, P<0.05) and baseline (overall +236±163 ml) (Figure 3C). Heparin (75,000 IU and 150,000 IU) also significantly (P<0.01) increased the change from baseline of trough FVC during the study period, compared with placebo. The effect of heparin administered at the higher dose was sustained and also greater at the follow-up visit (+105±213 ml vs. day 21, P>0.05) (Figure 3C and D). Conversely, heparin at the lower dose appeared to have a phasic effect on FEV_1 and FVC which were reduced at day 14 and day 28, compared to day 7 and 21 respectively.

As previously reported [31], water inhalation (7 ml), did not induce either an acute bronchoconstrictor or a broncholytic effect over the first 4h post administration during the study period. Heparin inhalation also did not induce any acute effect on lung function.

Impact of inhaled heparin on exercise capacity

Inhaled heparin significantly (P<0.05) increased the 6MWD, compared with the 6MWD covered at baseline (placebo -2±31, heparin 75,000 IU +85±38 m, and heparin 150,000 IU +123±26 m), and significantly (P<0.001) improved the post-exercise dyspnoea measured by the Borg score, compared with baseline (placebo -2.0±0.8, heparin 75,000 IU -3.8±0.4, and heparin 150,000 IU -3.1±0.5) (Figure 4A and B).

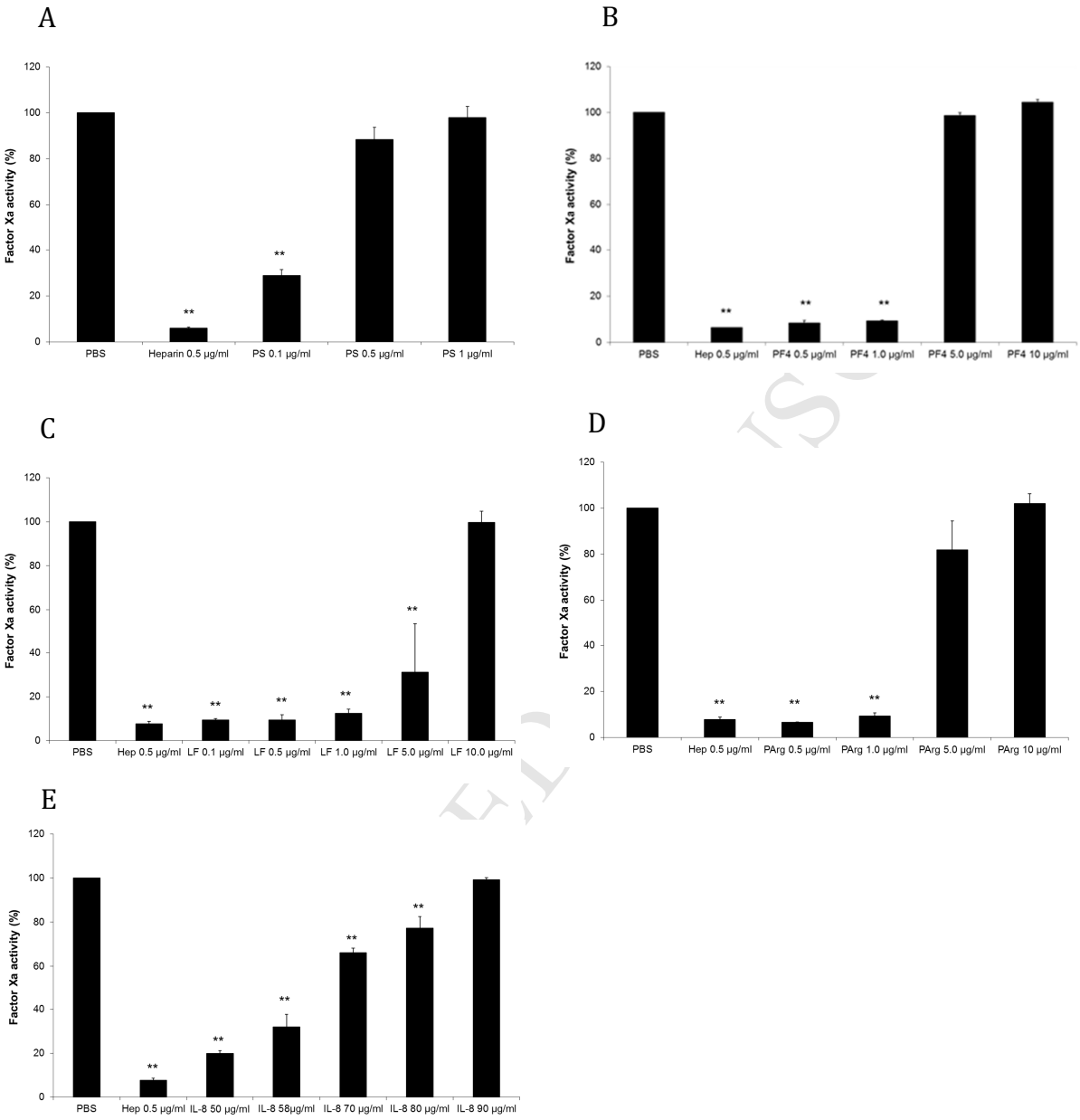
At day 21, inhaled heparin also improved the post-exercise SpO_2, compared with T0. The tendency of SpO_2 values from T0 to T21 was positive in patients receiving heparin. Indeed, the patient-by-patient analysis showed that in patients receiving heparin at the higher dose, this positive trend was significantly (P<0.05) different compared with the negative tendency of the placebo group (Figure 4C and D).

Impact of inhaled heparin on haematology and blood coagulation parameters

Overall, inhaled heparin did not negatively influence either haematology or blood coagulation parameters. At day 21, only the concentration of hemoglobin was significantly ($P<0.05$) higher in the arm of patients treated with heparin 75,000 IU compared with that of the placebo treated group (see Table E1 in the on-line supplement).

Adverse events

The administration of inhaled heparin at both low and high doses did not induce any serious adverse events. No significant ($P>0.05$) differences were detected with regard to adverse events such as cardiovascular, coagulation or respiratory disorders, between the patients treated with heparin at either dose and the placebo group (Table 2).


A
Factor Xa activity (%)
120
100
80
60
40
20
0
**
**
PBS
Heparin 0.5 µg/ml
PS 0.1 µg/ml
PS 0.5 µg/ml
PS 1 µg/ml
B
Factor Xa activity (%)
**
**
**
PBS
Hep 0.5 µg/ml
PF4 0.5 µg/ml
PF4 1.0 µg/ml
PF4 5.0 µg/ml
PF4 10 µg/ml
C
Factor Xa activity (%)
**
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**
PBS
Hep 0.5 µg/ml
LF 0.1 µg/ml
LF 0.5 µg/ml
LF 1.0 µg/ml
LF 5.0 µg/ml
LF 10.0 µg/ml
D
Factor Xa activity (%)
**
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**
PBS
Hep 0.5 µg/ml
PArg 0.5 µg/ml
PArg 1.0 µg/ml
PArg 5.0 µg/ml
PArg 10 µg/ml
E
Factor Xa activity (%)
**
**
**
**
**
PBS
Hep 0.5 µg/ml
IL-8 50 µg/ml
IL-8 58µg/ml
IL-8 70 µg/ml
IL-8 80 µg/ml
IL-8 90 µg/ml

Figure 1. The anticoagulant activity of heparin measured as inhibition of Factor Xa activity is neutralised by (A) protamine sulphate (PS), (B) platelet factor-4 (PF4), (C) lactoferrin (LF), (D) polyarginine (PArg) and (E) IL-8 in the concentration ranges shown. **; $p<0.01$ vs PBS control, n=3 for all except n=4 for PS.

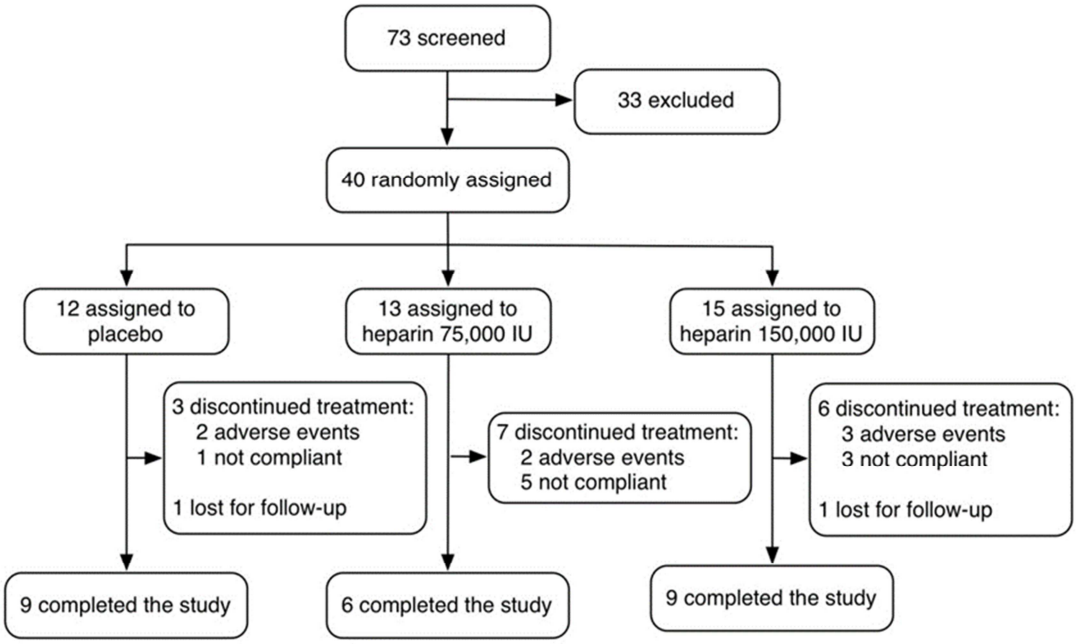

Figure 2. Study profile.

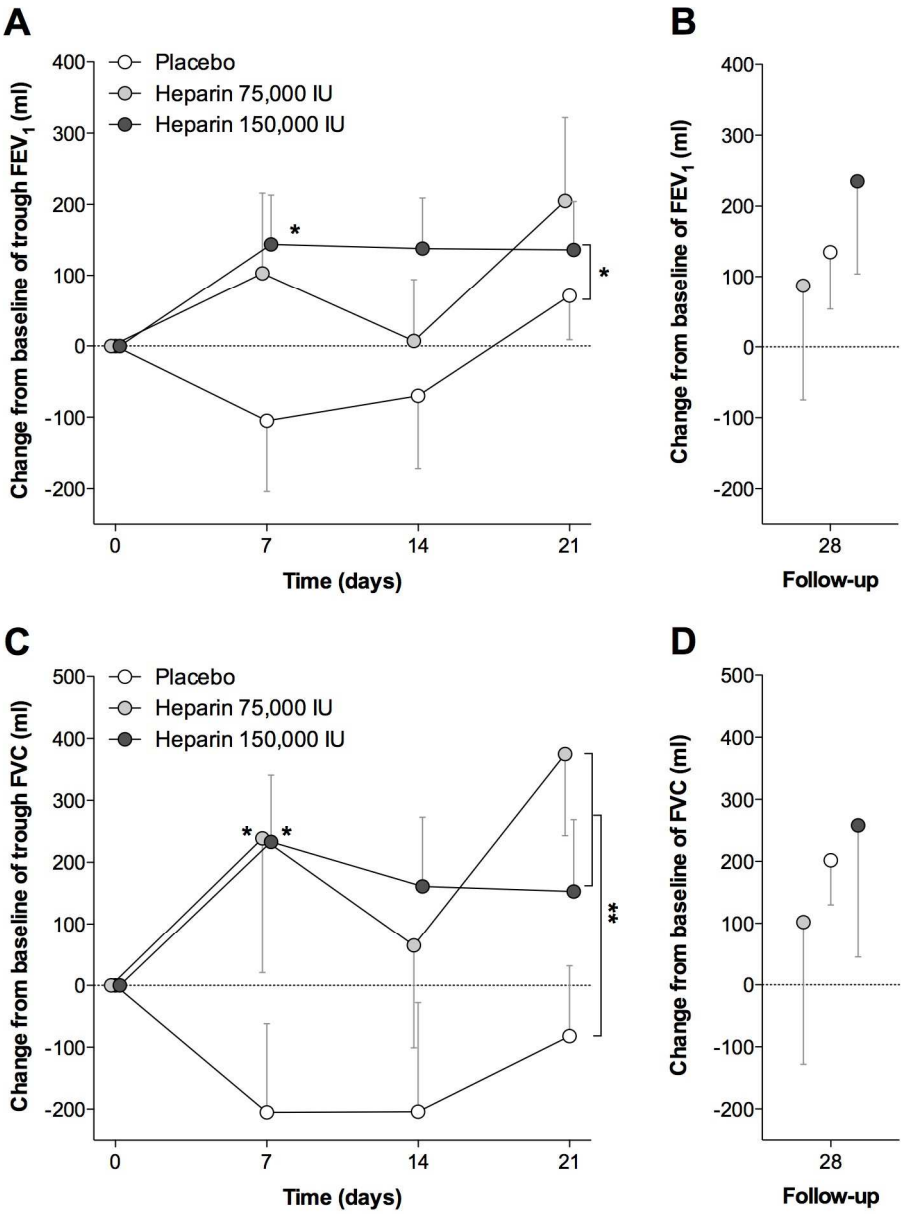

Figure 3. Influence of inhaled heparin on change from baseline of trough FEV_1 (upper panels) and FVC (lower panels) during the study period (A and C) and at follow-up (B and D). Data expressed as mean±SEM. * P<0.05 and ** P<0.01 vs. placebo. Statistical significance assessed by two-way ANOVA with Bonferroni post-test.

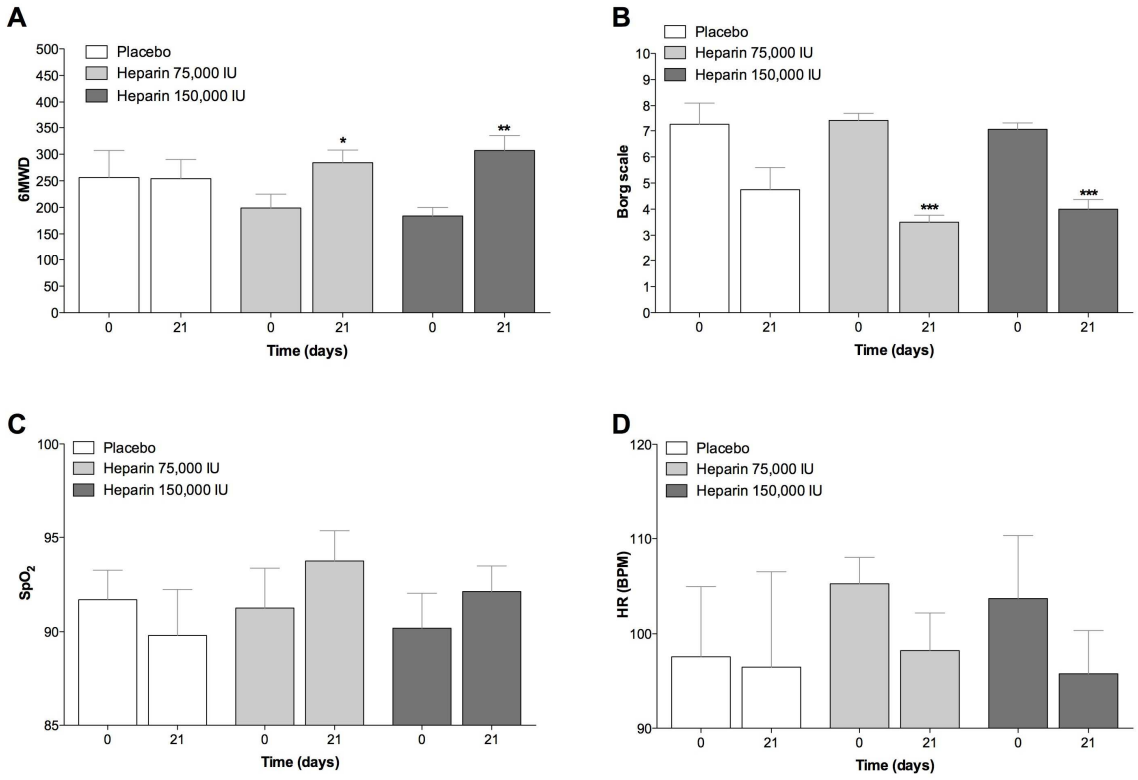

Figure 4. Influence of inhaled heparin on 6MWD (A), post-exercise Borg score (B), post-exercise SpO2 (C), and post-exercise heart rate (D). Data expressed as mean±SEM. * P<0.05, ** P<0.01 and *** P<0.001 vs. T0. Statistical significance assessed by t test.

Discussion

No clinical study to date has indicated adverse events or harm associated with the use of inhaled heparin [25, 26]. Our pre-clinical studies indicate this may be due to the presence of basic proteins with the capacity to neutralise the anticoagulant activity of UFH and the subsequent risk of hemoptysis in normal and inflamed airways.

Platelets are a rich source of PF-4, and platelets are known to be activated in COPD [32]. It has been known for a long time that PF-4 neutralises the anticoagulant activity of heparin in a 10:1 w:w ratio [27] and we confirmed this observation using the assay described above. Levels of ECP are high in COPD sputum, and ECP concentrations of 85.4 (49.6-319.5 µg/ml) are reported in stable COPD which increase significantly to 187.0 (81.3-295) on exacerbation [33]. Polyarginine, a model of arginine rich ECP, also neutralised the anti-coagulant activity of heparin in the same 10:1 w:w ratio as PF-4.

Lactoferrin is found in respiratory secretion at high concentrations (0.1 to 1.0 mg/ml) [34] and in COPD sputum at up to 300 µg/ml [35]. Lactoferrin at 10 µg/ml (20:1 w:w ratio with heparin) completely neutralised the anticoagulant activity of heparin, and on a molar basis, lactoferrin was nearly as potent as protamine sulphate. IL-8 is a chemokine present in high concentrations in COPD sputum [33]. IL-8 was present at 1.5 (0.9-4.4 µg/ml) in stable disease and this increased significantly to 7.78 (3.52-9.90) µg/ml on bacterial exacerbation. However, IL-8 was not as effective as PF4 in neutralising heparin anticoagulant activity and only at concentrations higher than 50 µg/ml was IL-8 able to neutralise the activity of heparin. However, the inflamed COPD airways contain multiple basic chemokines which overall may contribute to neutralising the anticoagulant activity of inhaled heparin [36].

The anticoagulant activity of heparin is associated with only one in three heparin chains and appears to play a minor role in many clinical uses and in physiological and pathological responses [37]. Our *in vitro* evidence is that the anti-coagulation activity of heparin is inhibited by basic proteins in sputum, leaving its other pharmacological anti-inflammatory and mucolytic activities, intact. Anti-coagulation effects are normally achieved at lower doses than either anti-inflammatory effects or mucolytic effects. Heparin dissolves F-actin and DNA bundles, activates DNase and reduces the viscosity of cystic fibrosis sputum [15]. We have observed the same effect of heparin on DNA in sputum from patients with COPD (unpublished observations). These effects are achieved at heparin concentrations of 1 mg/ml in sputum [15] and are not inhibited by basic proteins present at the concentrations indicated above, and are therefore independent of anticoagulant activity.

In our clinical study, inhaled nebulised UFH administered at 150,000 IU twice a day for 21 days significantly improved FEV_1, and both 75,000 IU and 150,000 IU twice a day for 21 days significantly improved FVC. The apparent biphasic effect of the lower dose may reflect the balance between achieving mucus clearance at this dose, and the uptake and sequestration of heparin [38] and large heparin/basic protein complexes [39] by pulmonary macrophages. At the higher dose the availability of heparin may not be limited to the same extent by this cellular storage mechanism and the effect is sustained.

Patients with moderate to severe COPD produce 20-100 ml sputum a day, (mean 39 ml [41]) which requires delivery of 20-100 mg heparin to the airway to deliver an effective (1 mg/ml) dose. In our study, heparin was delivered from a jet nebuliser, reported to deliver 8% [40] of the loading dose (150,000 IU, 750 mg) to the lungs i.e. 12,000 IU, 60 mg, to achieve a therapeutic concentration ~1.5 mg/ml in the airway. Effects of inhaled heparin on systemic coagulation were previously reported only for much higher doses (>8 mg/kg body weight) [38]. In parallel, our study found no effect of inhaled heparin at either dose on systemic coagulation parameters.

Pulmonary rehabilitation (PR) alone for 3 weeks had no effect on FEV_1 as we [29], and others [42], previously reported for PR programmes. Although improvements in post-exercise Borg score indicated improvement in dyspnoea, the 6MWD was not improved. Guidelines recommend 6-12 weeks of PR

therapy [43] and improvements in 6MWD are seen after these longer PR programmes [42]. However, more recently, intensive 7-days a week 3-week PR programmes were reported [44, 45] to significantly improve both FEV_1 and 6MWD in COPD patients with a wide range of disease severity. The addition of UFH at the highest dose for 7 days to PR resulted in a clinically significant improvement in FEV_1 of 144 ml from baseline, which was maintained over the course of the study and at follow-up.

All the patients in our trial were receiving concomitant medication with salbutamol (1 mg twice daily via nebulisation) and beclomethasone dipropionate (400 µg twice daily via nebulisation), as well as pulmonary rehabilitation. Mucus clearance devices were previously shown to increase response to bronchodilator therapy in COPD patients [46]. We speculate, therefore, that the beneficial effect of inhaled UFH observed in this study may be the result of the mucolytic activity of heparin [15], since mucolytic activity aids mucus clearance from the airways, reducing airway obstruction, and enhances bronchodilator/ICS delivery to target cells, with effects on lung function.

World-wide, the prevalence of patients with COPD GOLD stage II or higher is estimated at 10%, and a growing cause of morbidity and mortality [1]. It is clear that new therapeutic approaches are needed to alter the course of the disease, and the progressive loss of lung function leading to invalidity and death. Cough and sputum production are risk factors for poor outcomes in COPD [47] and it has been suggested that mucus hypersecretion should be a therapeutic target in all patients with COPD [48]. Recent meta-analyses indicate that mucolytics may reduce the number of exacerbations in patients with chronic bronchitis or COPD, and may have an effect on the duration and severity of exacerbations [3, 49, 50]. This is reflected in the most recent (2015) GOLD guidelines [3] indicating that exacerbations may be reduced by treatment with mucolytics such as N-acetyl cysteine, erdosteine and carbocysteine. It is not known, however, whether the clinical effect of these oral mucolytics also relates to their anti-oxidant properties [51].

Heparin administered at both doses significantly increased the 6MWD and reduced the post-exercise Borg score. The recommended minimal clinically important difference (MCID) is conservatively estimated to be 54–80 meters for the distance covered during the 6MWD assessment and 1-unit for the Borg score [52,53]. The change induced by both the doses of heparin in 6MWD indicated a clinically significant change in functional status [54] in the arms that received the active treatments, compared with the placebo group. Analogously, the improvement induced by heparin in the post-exercise Borg score suggested a clinically significant improvement in dyspnoea [55]. In fact, both doses of heparin reduced the Borg score of a further 1-unit as compared with the effect of the respiratory rehabilitation program detectable in the placebo group. Furthermore, heparin administered at the higher dose also significantly improved the post-exercise SpO_2 tendency, compared with placebo.

Bronchodilators improve the 6MWD [53] and the observed improvement is therefore likely to be related to inhaled heparin, alone or in combination with concomitant use of a bronchodilator.

UFH is a drug with a structurally diverse molecular scaffold naturally oriented to multiple targets, with mucolytic [15], wound healing, anti-inflammatory [14] and anti-oxidant [56] pharmacological activities when inhaled directly into the airways. The observed clinically significant improvements over a relatively short time in lung function, with improvements in patient-centred outcomes, in COPD may reflect this unique pharmacological profile. Although this RCT cannot define the mechanism of action of inhaled heparin, we hypothesise that the observed effects of inhaled heparin were at least partly due to the mucolytic properties of heparin, as well as the anti-inflammatory effects of heparin [14], including inhibition of neutrophil elastase [57], a potent mucus secretagogue and elastolytic inducer of emphysema [36, 58]. Further, it has been hypothesised that afferent C-fibres are stimulated by inflamed airways in COPD, and play a role in excessive mucus secretion, airways obstruction, rapid shallow breathing, skeletal myopathy and CO2 retention, especially during acute exacerbations [59]. In this case we can speculate that inhaled heparin neutralised the cationic proteins, including ECP, capable of C-fibre stimulation [60].

Although pre-clinical studies have indicated the long-term safety of inhaled heparin [38] further clinical studies are needed to confirm the long term safety of inhaled heparin in patients with COPD.

Concluding, the results of this RCT indicate that inhaled nebulised UFH improves the pulmonary function, exercise capacity and dyspnoea in patients with moderate to very severe COPD, with no significant changes in blood coagulation parameters, and a good safety profile.

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References

1. Buist AS, McBurnie MA, Vollmer WM, Gillespie S, Burney P, Mannino DM, Menezes AM, Sullivan SD, Lee TA, Weiss KB, Jensen RL, Marks GB, Gulsvik A,Nizankowska-Mogilnicka E; BOLD Collaborative Research Group. International variation in the prevalence of COPD (the BOLD Study): a population-based prevalence study. Lancet. 2007;370:741-50.

2. Cazzola M, Rogliani P, Ora J, Matera MG. Treatment options for moderate-to-very severe chronic obstructive pulmonary disease. Expert Opin Pharmacother. 2016;17:977-88.

3. GOLD. Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD, updated 2015). Available from: http://www.goldcopd.org/. 2015.

4. Cazzola M, Matera MG. Bronchodilators: current and future. Clin Chest Med. 2014;35:191-201.

5. Calzetta L, Matera MG, Cazzola M. Pharmacological interaction between LABAs and LAMAs in the airways: optimizing synergy. Eur J Pharmacol. 2015;761:168-73.

6. Matera MG, Cardaci V, Cazzola M, Rogliani P. Safety of inhaled corticosteroids for treating chronic obstructive pulmonary disease. Expert Opin Drug Saf. 2015;14:533-41.

7. Rogliani P, Calzetta L, Cazzola M, Matera MG. Drug safety evaluation of roflumilast for the treatment of COPD: a meta-analysis. Expert Opin Drug Saf. 2016; 20:1-14.

8. Cazzola M, Page C, Calzetta L, Matera MG. Pharmacology and therapeutics of bronchodilators. Pharmacol Rev. 2012;64:450-504.

9. Cazzola M, Page CP, Calzetta L, Matera MG. Emerging anti-inflammatory strategies for COPD. Eur Respir J. 2012;40:724-41.

10. Page C, Cazzola M. Bifunctional drugs for the treatment of asthma and chronic obstructive pulmonary disease. Eur Respir J. 2014;44:475-82.

11. Matera MG, Calzetta L, Cazzola M. Oxidation pathway and exacerbations in COPD: the role of NAC. Expert Rev Respir Med. 2016;10:89-97.

12. Zheng JP, Kang J, Huang SG, Chen P, Yao WZ, Yang L, Bai CX, Wang CZ, Wang C, Chen BY, Shi Y, Liu CT, Chen P, Li Q, Wang ZS, Huang YJ, Luo ZY, Chen FP, Yuan JZ, Yuan BT, Qian HP, Zhi RC, Zhong NS. Effect of carbocisteine on acute exacerbation of chronic obstructive pulmonary disease (PEACE Study): a randomised placebo-controlled study. Lancet. 2008 14;371:2013-8.

13. Dal Negro R, Wedzicha, J, Iversen M, Fontana G, page CP, Cicero A, Pozzi, E and Calverley P. Effect of erdosteine on the rate and duration of COPD exacerbations: The RESTORE study. Eur Resp J 2017; in press.

14. Mulloy B, Hogwood J, Gray E, Lever R, Page CP. Pharmacology of heparin and related drugs. Pharmacol Rev 2016; 68: 76-141.

15. Broughton-Head VJ, Shur J, Carroll MP, Smith JR, Shute JK. Unfractionated heparin reduces the elasticity of sputum from patients with cystic fibrosis. Am J Physiol Lung Cell Mol Physiol. 2007;293:L1240-9.

16. Capila I, Linhardt RJ. Heparin-protein interactions. Angew Chem Int Ed Engl. 2002;41:391-412.

17. Brown RA, Allegra L, Matera MG, Page CP, Cazzola M. Additional clinical benefit of enoxaparin in COPD patients receiving salmeterol and fluticasone propionate in combination. Pulm Pharmacol Ther. 2006;19:419-24.

18. Bendstrup KE, Gram J, Jensen JI. Effect of inhaled heparin on lung function and coagulation in healthy volunteers. Eur Respir J 2002; 19: 606-610.

19. Markart P, Nass R, Ruppert C, Hundack L, Wygrecka M, Korfei M, Boedeker RH, Staehler G, Kroll H, Scheuch G, Seeger W, Guenther A. Safety and tolerability of inhaled heparin in idiopathic pulmonary fibrosis. J Aerosol Med Pulm Drug Deliv. 2010;23:161-72.

20. Diamant Z, Timmers MC, van der Veen H, Page CP, van der Meer FJ, Sterk PJ. Effect of inhaled heparin on allergen-induced early and late asthmatic responses in patients with atopic asthma. Am J Respir Crit Care Med. 1996;153:1790-5.

21. Dixon B, Santamaria JD, Campbell DJ. A phase 1 trial of nebulised heparin in acute lung injury. Crit Care. 2008;12:R64.

22. Garrigo J, Danta I, Ahmed T. Time course of the protective effect of inhaled heparin on exercise-induced asthma. Am J Respir Crit Care Med. 1996;153:1702-7.

23. Serisier DJ, Shute JK, Hockey PM, Higgins B, Conway J, Carroll MP. Inhaled heparin in cystic fibrosis. Eur Respir J. 2006;27:354-8.

24. Youngchaiyud P, Kettel LJ, Cugell DW. The effect of heparin aerosols on airway conductance in patients with chronic obstructive pulmonary disease. Am Rev Respir Dis. 1969;99:449-52.

25. Monagle K, Ryan A, Hepponstall M, Mertyn E, Monagle P, Ignjatovic V, Newall F. Inhalational use of antithrombotics in humans: Review of the literature. Thrombosis Res 2015;136:1059-1066.

26. Mousavi S, Moradi M, Khorshidahmad T, Motamedi M. Anti-Inflammatory Effects of Heparin and Its Derivatives: A Systematic Review. Adv Pharmacol Sci. 2015;2015:507151.

27. Denton J, Lane DA, Thunberg L, Slater AM, Lindahl U. Binding of platelet factor 4 to heparin oligosaccharides. Biochem J 1983; 209: 455-460.

28. Ramos FL, Krahnke JS, Kim V. Clinical issues of mucus accumulation in COPD. Int J COPD 2014; 9: 139-150.

29. Pasqua F, Biscione G, Crigna G, Auciello L, Cazzola M. Combining triple therapy and pulmonary rehabilitation in patients with advanced COPD: a pilot study. Respir Med. 2010 Mar;104(3):412-7.

30. Cazzola M, MacNee W, Martinez FJ, Rabe KF, Franciosi LG, Barnes PJ, Brusasco V, Burge PS, Calverley PM, Celli BR, Jones PW, Mahler DA, Make B, Miravitlles M, Page CP, Palange P, Parr D, Pistolesi M, Rennard SI, Rutten-van Mölken MP, Stockley R, Sullivan SD, Wedzicha JA, Wouters EF. Outcomes for COPD pharmacological trials: from lung function to biomarkers. Eur Respir J. 2008;31:416-69.

31. Malik SK, Jenkins DE. Alterations in airway dynamics following inhalation of ultrasonic mist. Chest; 62: 660-664.

32. Pitchford SC. Novel uses for anti-platelet agents as anti-inflammatory drugs. Br J Pharmacol 2007; 152: 987-1002.

33. Bathoorn E, Liesker JJW, Postma DS, Koëter GH, van der Toorn M, van der Heide S, Ross HA, van Oosterhout AJ, Kerstjens HA. Change in inflammation in out-patient COPD patients from stable phase to a subsequent exacerbation. Int J COPD 2009; 4: 101-109.

34. Rogan MP, Geraghty P, Greene CM, O'Neill SJ, Taggart CC, McElvaney NG. Antimicrobial proteins and polypeptides in pulmonary innate defence. Resp Res 2006; 7: 29

35. Parameswaran GI, Sethi S, Murphy TF. Effects of bacterial infection on airway antimicrobial peptides and proteins in COPD. Chest 2011; 140: 611-617.

36. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 2016; 138: 16-27.

37. Jacques LB. Heparin: an old drug with a new paradigm. 1979; 206: 528-533.

38. Jaques LB, Mahadoo J, Kavanagh LW. Intrapulmonary heparin. A new procedure for anticoagulant therapy. Lancet. 1976;308:1157-61.

39. Joglekar M, Khandelwal S, Cines DB, Poncz M, Rauova L, Arepally GM. Heparin enhances uptake of platelet factor 4/heparin complexes by monocytes and macrophages. J Thromb Haemost. 2015;13:1416-27.

40. Bendstrup KE, Chambers CB, Jensen JI, Newhouse MT. Lung deposition andclearance of inhaled (99m)Tc-heparin in healthy volunteers. Am J Respir Crit CareMed. 1999;160:1653-8.

41. Chakravorty I, Chahal K, Austin G. A pilot study of the impact of high-frequency chest wall oscillation in chronic obstructive pulmonary disease patients with mucus hypersecretion. Int J COPD 2011; 6: 693-699.

42. Mulhall P, Criner G. Non-pharmacological treatments for COPD. Respirology 2016; 21: 791-809.

43. BTS guidelines on pulmonary rehabilitation in adults. Thorax 2013, 68,S2.

44. Santus P, Radovanovic D, Balzano G, Pecchiari M, Raccanelli R, Sarno N, DiMarco F, Jones PW, Carone M. Improvements in Lung Diffusion Capacity following Pulmonary Rehabilitation in COPD with and without Ventilation Inhomogeneity. Respiration. 2016;92(5):295-307.

45. Greulich T, Koczulla AR, Nell C, Kehr K, Vogelmeier CF, Stojanovic D, Wittmann M, Schultz K. Effect of a Three-Week Inpatient Rehabilitation Program on 544 Consecutive Patients with Very Severe COPD: A Retrospective Analysis.Respiration. 2015;90(4):287-92.

46. Wolkove N, Baltzan MA, Kamel H, Rotaple M. A randomized trial to evaluate the sustained efficacy of a mucus clearance device in ambulatory patients with chronic obstructive pulmonary disease. Can Respir J 2004; 11: 567-572.

47. Miravitlles M. Cough and sputum production as risk factors for poor outcomes in patients with COPD. Respir Med. 2011;105:1118-28.

48. Martin C, Frija-Masson J, Burgel P-R. Targeting mucus hypersecretion: new therapeutic opportunities for COPD? Drugs 2014; 74: 1073-1089

49. Poole P, Chong J, Cates CJ. Mucolytic agents versus placebo for chronic bronchitis or chronic obstructive pulmonary disease. Cochrane Database Syst Rev. 2015;(7):CD001287.

50. Cazzola M, Calzetta L, Page C, Jardim J, Chuchalin AG, Rogliani P, Matera MG. Influence of N-acetylcysteine on chronic bronchitis or COPD exacerbations: a meta-analysis. Eur Respir Rev. 2015;24:451-61.

51. Rahman I, MacNee W. Antioxidant pharmacological therapies for COPD. Curr Opin Pharmacol. 2012;12:256-65.

52. Ries AL. Minimally clinically important difference for the UCSD Shortness of Breath Questionnaire, Borg Scale, and Visual Analog Scale. COPD. 2005;2:105-10.

53. Wise RA, Brown CD. Minimal clinically important differences in the six-minute walk test and the incremental shuttle walking test. COPD. 2005;2:125-9.

54. Laboratories ATSCoPSfCPF. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166:111-7.

55. Eaton T, Garrett JE, Young P, Fergusson W, Kolbe J, Rudkin S et al. Ambulatory oxygen improves quality of life of COPD patients: a randomised controlled study. Eur Respir J. 2002;20:306-12.

56. Grant D, Long WF, Mackintosh G, Williamson FB. The antioxidant activity of heparins. Biochem Soc Trans. 1996;24:194S.

57. Redini F, Tixier JM, Petitou M, Choay J, Robert L, Hornebeck W. Inhibition of leucocyte elastase by heparin and its derivatives. Biochem J. 1988;252:515-9.

58. Demkow U, van Overveld FJ. Role of elastases in the pathogenesis of chronic obstructive pulmonary disease: implications for treatment. Eur J Med Res. 2010; 4;15 Suppl 2:27-35.

59. Macklem PT. Therapeutic implications of the pathophysiology of COPD. Eur Respir J 2010; 35: 676-680.

60. Lee L-Y, Gu Q. Mechanisms of bronchopulmonary C-fiber hypersensitivity induced by cationic proteins. Pulmon Pharmacol Therapeut 2003; 16: 15-22.

Tables

Table 1. Patient demographic and baseline characteristics.

	Placebo (n=12)	Heparin 75000 IU (n=13)	Heparin 150000 IU (n=15)	Overall (n=40)
Age (years)	71.55±1.59	72.54±2.46	72.00±2.30	72.05±1.25
Male/female	9/3	9/4	9/6	27/13
Current smokers (n)	3	4	3	10
Pack-years	56.39±10.34	56.93±9.35	45.87±7.53	56.65±5.12
C-reactive protein (mg/L)	3.01±1.81	2.26±.98	1.20±0.55	2.10±0.67
FEV_1 (%predicted)	50.45±3.12	48.57±2.25	41.53±2.51 *	46.50±1.60
FEV_1 (L)	1.24±0.13	1.21±0.09	0.97±0.07	1.13±0.06
FEV_1/FVC (%)	52.9±3.6	50.9±2.4	48.6±2.7	50.6±1.7

Data expressed as mean±SEM. * P<0.05 vs. placebo.

Table 2. Summary of adverse events.

	Placebo	Heparin 75,000 IU	Heparin 150,000 IU
Serious adverse events	0	0	0
Further adverse events:			
Cardiovascular disorders			
Hypotension	0	1	0
Coagulation disorders			
Epistaxis	1	1	1
Hemoptysis	0	0	1
Respiratory disorders			
COPD exacerbation	1	0	0
Pneumonia	0	0	1
Others			
Tibial fracture	0	1	0