Accepted Manuscript

Title: Therapeutic use of heparin and derivatives beyond anticoagulation in patients with bronchial asthma or COPD

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PII: S1471-4892(17)30228-X
DOI: https://doi.org/doi:10.1016/j.coph.2018.01.006
Reference: COPHAR 1700

To appear in: *Current Opinion in Pharmacology*

Received date: 22-12-2017
Revised date: 23-1-2018
Accepted date: 25-1-2018

Please cite this article as: Shute JK, Puxeddu E, Calzetta L, Therapeutic use of heparin and derivatives beyond anticoagulation in patients with bronchial asthma or COPD, *Current Opinion in Pharmacology* (2018), https://doi.org/10.1016/j.coph.2018.01.006

Therapeutic use of heparin and derivatives beyond anticoagulation in patients with bronchial asthma or COPD

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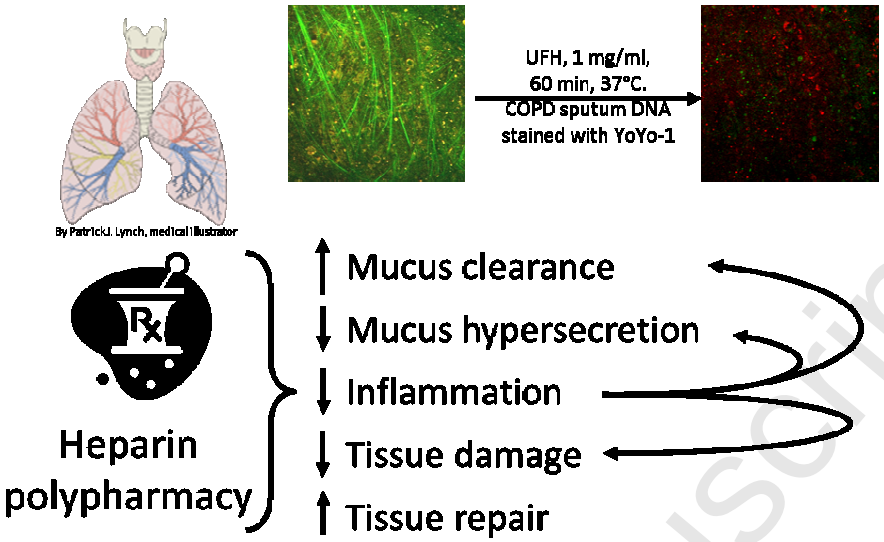
Word count: 2536

Abstract

In this review we identify potential targets for the therapeutic effects of heparin in asthma and chronic obstructive pulmonary disease (COPD), consider the safety and delivery modalities of this therapeutic approach. Specifically, we point to the anti-inflammatory, antioxidant and mucolytic effects of unfractionated heparin with potential to modify disease progression in COPD and asthma when administered via the inhaled route. Inhaled heparin may represent an effective add-on therapy in COPD and asthma patient groups, especially when taking into consideration the relative deficiency in endogenous heparin reported in asthma patients



Highlights

- Heparin is a unique molecule in biology with multiple pharmacological properties
- Heparin therapy has benefits in patients with COPD and asthma
- Inhaled heparin is safe and without adverse events associated with anti-coagulation

Introduction

The pathogenesis of asthma and COPD

Asthma and chronic obstructive pulmonary disease (COPD) are clinical conditions affecting millions of people world-wide [1]. The prevalence of COPD is increasing globally and it is predicted to be the third major cause of death world-wide by 2020, with over 3 million deaths due to COPD in 2012 [1]. Asthma and COPD are characterised by chronic bronchial airway inflammation and mucus hypersecretion which are associated with airflow limitation. In asthma, airflow is acutely limited, reversibly, by mast cell activation and release of mediators of bronchoconstriction, while there is limited evidence for mast cell activation contributing to the irreversible airflow limitation in COPD [2]. In COPD, airflow is further limited by small airway inflammation and fibrosis, as well as emphysema and gas trapping in the lung. The aetiology of these diseases is associated with environmental risk factors such as allergens and pollutants in asthma, and cigarette smoking, indoor smoke particles and outdoor pollution in COPD, interacting with susceptibility genes [3, 4]. Acute exacerbation

of symptoms requiring hospitalisation are predominantly induced by rhinovirus (RV) infection of the respiratory tract as well as allergen exposure in asthma, while in COPD exacerbations are predominantly caused by viral (RV, respiratory syncytial virus and influenza virus) or bacterial infections or both [5].

The inflammatory cell profile in asthma is typically eosinophilic and driven by a Th2 (IL-4, IL-13, IL-5) cytokine profile that is responsive to corticosteroid therapy. However, neutrophilic inflammation in the airway is also present during exacerbation and is associated with asthma severity and resistance to corticosteroid therapy. Neutrophilic inflammation in asthma has been associated with TH1 (IFNγ), Th17 (IL-17) and IL-8 expression and combined eosinophilic/neutrophilic inflammation may be a biomarker of the most severe form of asthma [6].

Airway inflammation in stable COPD is characterised by increased numbers of macrophages, neutrophils, dendritic cells, T-cells and B-cells, and increased numbers of airway neutrophils correlate with disease severity. COPD exacerbations induced by bacteria or viruses are accompanied by increased numbers of neutrophils in the airway driven by an increase in IL-8 concentrations in the airway, while the additional presence of airway eosinophils is associated with viral infections and increased Th2 cytokine production [7].

Oxidative stress is a feature of bronchial asthma [8] and COPD, with wide-ranging effects in the airways and lung parenchyma in COPD [9]. Reactive oxygen species (ROS) may be from exogenous sources such as pollution and cigarette smoke, as well as endogenous sources including activated neutrophils and eosinophils, and ROS drive further inflammation. Multiple biological effects of ROS include activation of the pro-inflammatory transcription factor NF-kB, activation of histone acetyl transferase and inhibition of histone deacetylase2 (HDAC2) which together enhance inflammatory gene transcription and corticosteroid resistance. Further effects of ROS include oxidative damage to proteolytic defences such as the elastase inhibitors alpha1-antitrypsin and secretory leukocyte protease inhibitor (SLPI), pathological changes in respiratory epithelial cells and mucus hypersecretion [8, 9].

Genetic and acquired defects in airway epithelial cell function have been described in asthma [10] and COPD [11], respectively. Defective epithelial barrier function in asthma is the result of the epithelium being both inherently fragile and susceptible to damage by exogenous pathogens, ROS, allergens and particulate matter, as well as endogenous eosinophil-derived cationic proteins [12], while having an impaired repair response [10]. Oxidative and nitrosative stress may play a role in elastase-induced lung tissue damage and emphysema [13] with genetic and acquired defects in antioxidant function contributing to the role of oxidative stress in COPD [14].

Mucus hypersecretion is a feature of many patients with asthma [10] and many, if not all, patients with COPD, in whom mucus hypersecretion increases with airflow limitation but is not always associated with chronic cough and sputum production [15]. EGFR-mediated goblet cell hyperplasia is a feature of both asthma and COPD [9, 10], with increased secretion of mucus containing the gel-forming mucin MUC5AC in response to EGFR-mediated activation of goblet cells by endogenous ligands (EGF, TGFα), ROS and IL-13 and

MUC5 from mucus glands [16]. Neutrophil elastase is a potent activator of EGFR via cleavage of the membrane-anchored pro-TGFα from the epithelial surface and release of mature TGFα which binds and activates EGFR and mucin synthesis [9]. Following secretion, mucus may be relatively dehydrated, making it difficult to clear, as a consequence of acquired defects in the cystic fibrosis transmembrane conductance regulator (CFTR) protein function in both COPD and, more controversially, in asthma [11].

Neutrophil elastase plays a major role in the pathogenesis of COPD and severe asthma not only as a mediator of mucus hypersecretion, but in driving the protease/antiprotease imbalance through proteolytic inactivation of the tissue inhibitor of metalloproteinases-1 (TIMP-1) and SLPI, a potent inhibitor of elastase activity [6,9]. Increased elastase activity in COPD contributes to tissue damage and emphysema, as well as further cycles of neutrophilic inflammation by augmenting epithelial IL-8 expression and generating neutrophil chemoattractant matrikines [9]. Neutrophil elastase activity cripples the immune response and mucociliary clearance in patients with cystic fibrosis [17], and may similarly be responsible for defective mucus clearance in asthma and COPD, mucus obstruction of the airways and susceptibility to infection.

Neutrophil elastase is found in association with neutrophil extracellular traps (NETs). NETs are formed from decondensed nuclear DNA, released in response to generation of intracellular ROS and elastase-mediated degradation of nuclear histones, which bind antimicrobial factors to form a physical and chemical barrier to the spread of pathogens. NETs are involved in the elimination of pathogens however, in excess, they also contribute to airway obstruction and tissue damage in both asthma and COPD [6, 18]. IL-8 is a potent NET inducer and NETs are present in COPD patients with both stable disease and during acute exacerbations, and correlate with the severity of airflow limitation [18]. Eosinophil extracellular traps (EETs) are also present in both asthmatic and COPD airways, and may be composed of nuclear or mitochondrial DNA [18, 19]. Further, it has recently been suggested that early formation of EETs triggers disease pathogenesis in COPD [19]. NETs and EETs are therefore therapeutic targets in asthma and COPD.

Heparin

Heparin is a member of the glycosaminoglycan family of linear, anionic, polysaccharides consisting of repeating disaccharide units, with variable degrees of sulphation and therefore considerable microheterogeneity in the structure [20]. Its biological synthesis generates highly diverse polysaccharide chains, only a third of which contain the pentasaccharide sequence responsible for binding and activation anti-thrombin III (ATIII) and the anti-coagulation properties of heparin. Medicinal heparin is derived from pig intestinal mucosa [21] and has an average molecular weight range of 12-16,000 for the size-unfractionated heparin. Low molecular weight heparins are derived from unfractionated heparin by various chemical processes generating heparins with average molecular weights from 3,600 to 6,500 depending on the process [22].

The multiple pharmacological effects of heparin

Heparin has the highest negative charge of any molecule in biology and multiple protein binding partners [20]. Many of these interactions are charge-dependent although in some cases a specific saccharide protein-binding sequence has been determined (as for AT-III). As a result, heparin has multiple pharmacological properties beyond anti-coagulation that are independent of its anti-coagulant activity [20, 23]. These include the anti-inflammatory effects of heparin and inhibition of each of the steps involved in inflammatory cell recruitment; inhibition of neutrophil activation and degranulation, inhibition of the steps leading to transendothelial migration of neutrophils, including rolling and adhesion, platelet/leukocyte interactions and inhibition of heparanase activity [20]. The major chemokine directing neutrophil recruitment into lung tissue is IL-8 bound to endothelial cell-surface proteoglycans. Heparin displaces, binds and inhibits the chemotactic activity of IL-8 by forming a complex with IL-8 that is unable to activate its receptor [24].

These anti-inflammatory effects are likely to combine to reduce the numbers of activated neutrophils in the lung, and consequently the burden of neutrophil elastase activity and NETs in the airway. Additionally, unfractionated heparin and low molecular weight heparin (LMWH) are equally able to inhibit elastase release from activated neutrophils, and even shorter saccharide chains derived from LMWH, with a minimum chain length of ten saccharides, are also effective [25]. However, the short chain saccharides and the LMWH from which they are derived were not effective inhibitors of neutrophil adhesion to endothelial cells [25]. Further, pre-clinical studies have indicated that unfractionated heparin is a considerably more potent local anti-inflammatory agent than low molecular weight heparin (dalteparin sodium) or a selectively 2 and 3-O desulphated non-anticoagulant derivative of heparin [26].

Further, heparin has direct inhibitory effects on neutrophil elastase and cathepsin G that correlate positively with the saccharide chain length and degree of sulphation [27]. In view of the fact that elastase is a potent mucus secretagogue and tissue elastolytic enzyme there are multiple beneficial effects of reducing the elastase burden in COPD and severe asthma, which should overall restore mucociliary clearance and the protease/antiprotease balance, while limiting tissue damage. Delivery of exogenous protease inhibitors for the treatment of COPD has been proposed to have many advantages [28], and inhaled unfractionated heparin may be a promising candidate.

The anti-oxidant activity of heparin [29] may further reduce inflammation, protect anti-proteases from oxidative inactivation and limit ROS-induced mucus hypersecretion. In COPD, severe asthma and asthmatics who smoke, HDAC2 is reduced, preventing corticosteroids from inactivating activated inflammatory genes. The reduction in HDAC2 appears to be secondary to increases in oxidative and nitrosative stress in the lungs [30]. Antioxidants and inhibitors of nitric oxide synthesis, such as heparin [31], may therefore restore corticosteroid sensitivity in COPD.

The eosinophil cationic proteins major basic protein (MBP) and eosinophil cationic protein (ECP) are elevated in airway secretions in asthma, and found in association with EETs [12]. Although these basic proteins have roles in host defence against a range of pathogens, they are highly cytotoxic, and induce bronchoconstriction and airway hyperresponsiveness.

Heparin neutralises the charge on eosinophil basic proteins and inhibits these effects [12, 32]. Heparin may also inhibit ECP in the inflamed airways and alveoli that is responsible for C-fibre stimulation and mucus hypersecretion in asthma and COPD [33, 34].

Mucus hypersecretion and obstruction of the airways is compounded by the presence and NETs and EETs in asthma and COPD sputum. DNA contributes to sputum elasticity and reduced cough clearance, and in cystic fibrosis (CF) sputum we showed that heparin disaggregates DNA/actin bundles and activates endogenous DNase to reduce sputum elasticity [35]. We see the same effects in sputum from patients with COPD [unpublished, but see the graphical abstract], and this is predicted to improve cough clearance in COPD. We also showed that when DNase I breaks down DNA, IL-8 encrypted by the DNA is released and induces increased neutrophil migration *in vitro* [36]. Together with release of elastase activity [18], this pro-inflammatory effect of DNase alone would potentially be avoided by co-administration of DNase I with heparin by inhalation.

Electrostatic mucin interactions and viscosity are increased by a low pH in airway surface liquid, as seen in asthma and COPD, and these effects are reversed by heparin [37]. Further, oxidation increases mucin cross-links and the elasticity of sputum [38]. These effects, together with the presence of DNA, may contribute to the abnormal viscoelasticity of sputum from patients with asthma [39]. Thiol antioxidants such as N-acetyl-L-cysteine (NAC), carbocisteine and erdosteine are oral mucolytic drugs that at high doses may improve the number of exacerbations in COPD, without impact on airflow obstruction [13, 40-43]. However, despite the fact that mucus accumulation is a major factor in airway obstruction in asthma, no effective mucolytic treatments for asthma exist. We suggest that inhaled heparin, through inhibition of mucus hypersecretion and effects on mucus rheology, may be an effective mucoregulatory agent and mucolytic drug in asthma and COPD. In support of this notion, unfractionated and LMWH inhibited endotoxin and allergen-induced nasal mucus secretion and MUC5AC expression by airway epithelial cells directly and indirectly, through suppression of neutrophilic and eosinophilic inflammation, in pre-clinical models [44, 45].

The multiple effects of inhaled unfractionated heparin are illustrated in Figure 1.

Safety and delivery modalities

While we and others [46, 47] have reported coagulation and fibrin formation in the asthmatic airways, the positive effects of inhaled unfractionated heparin in clinical studies in asthma [48, 49] and COPD [50] are unlikely to be associated with the anticoagulant properties of heparin as these are neutralised by the plethora of basic proteins present at elevated concentrations in inflamed airways in asthma and COPD [50]. Further, intrapulmonary heparin does not cross the bronchial mucosa when nebulised at doses up to 8 mg/kg body weight and no acute or chronic toxicological effects were reported in any species, or at any dose, studied [48-51]. The delivered lung dose is determined by the nebuliser efficiency and the concentration by the volume of secretions into which heparin is delivered. Bendstrup et al. [52] demonstrated that 8% of the loading dose is delivered to the lung from a jet nebuliser and that a lung dose of 32,000 International Units (~160 mg)

to the lower respiratory tract can safely be inhaled for clinical or research purposes. In COPD, we have estimated a safe and effective lung dose of 60 mg [50].

Since anticoagulation is associated with intravenous and subcutaneous administration of heparin, the inhaled route may be safe and effective in asthmatic and COPD patients. Further, since systemic delivery and anti-coagulation was achieved by inhalation of dry particles of heparin and LMWH [53] or as a liquid instilled via the nasal route [54], then we believe that nebulisation and oral inhalation of an aerosol of liquid droplets of unfractionated heparin solution is the preferred delivery modality.

In addition to mucolytic and mucoregulatory effects that are predicted to reduce airway obstruction and improve airflow, nebulised unfractionated heparin is proposed to have anti-inflammatory, anti-oxidant and anti-nitrosative effects that protect the airways and lung tissue from damage, as well as reparative effects to restore functional airway epithelium [20, 55].

Clinical trials of heparin in asthma and COPD

Monagle et al. [48] reviewed 14 studies of inhaled unfractionated and LMWH in adults and children with asthma and allergy. These studies reported positive effects of inhaled heparin on lung function in allergen and exercise induced asthma, and reduced bronchoconstriction following provocation with water, methacholine, adenosine, histamine and hypertonic KCl, with no systemic anticoagulation or adverse effects such as pulmonary haemorrhage.

Table 1 summarises studies investigating the effect of heparin and LMWH in patients with COPD. Positive effects on lung function were seen in all studies, whether heparin was administered via the inhaled, subcutaneous or intravenous route. In our RCT of inhaled unfractionated heparin in patients with moderate to severe COPD we observed clinically significant improvements over a relatively short time in lung function, exercise capacity and dyspnea [50].

Conclusion

Unfractionated heparin is a drug with a structurally diverse molecular scaffold naturally orientated to multiple molecular targets with mucolytic, anti-inflammatory, anti-oxidant and wound healing properties. These effects are independent of anti-coagulation activity when heparin is inhaled directly into the airways.

Currently, there are no clinically available therapies that prevent COPD disease progression. Inhaled heparin may therefore represent an effective add-on therapy in COPD and asthma patient groups, especially when taking into consideration the relative deficiency in endogenous heparin reported in asthma patients [20]. Overall, we believe that the multiple pharmacological effects of inhaled unfractionated heparin are likely to have greater therapeutic benefit in patients with asthma and COPD than compounds acting against a single target.

Acknowledgements

This research did not receive any specific grant from any funding agency.

Declaration of interest

Janis Shute is the Scientific Director of Ockham Biotech Ltd that holds granted patents around the use of inhaled heparin. Luigino Calzetta acted as a consultant for Ockham Biotech Ltd. Ermanno Puxxeddu has no conflict if interest to declare.

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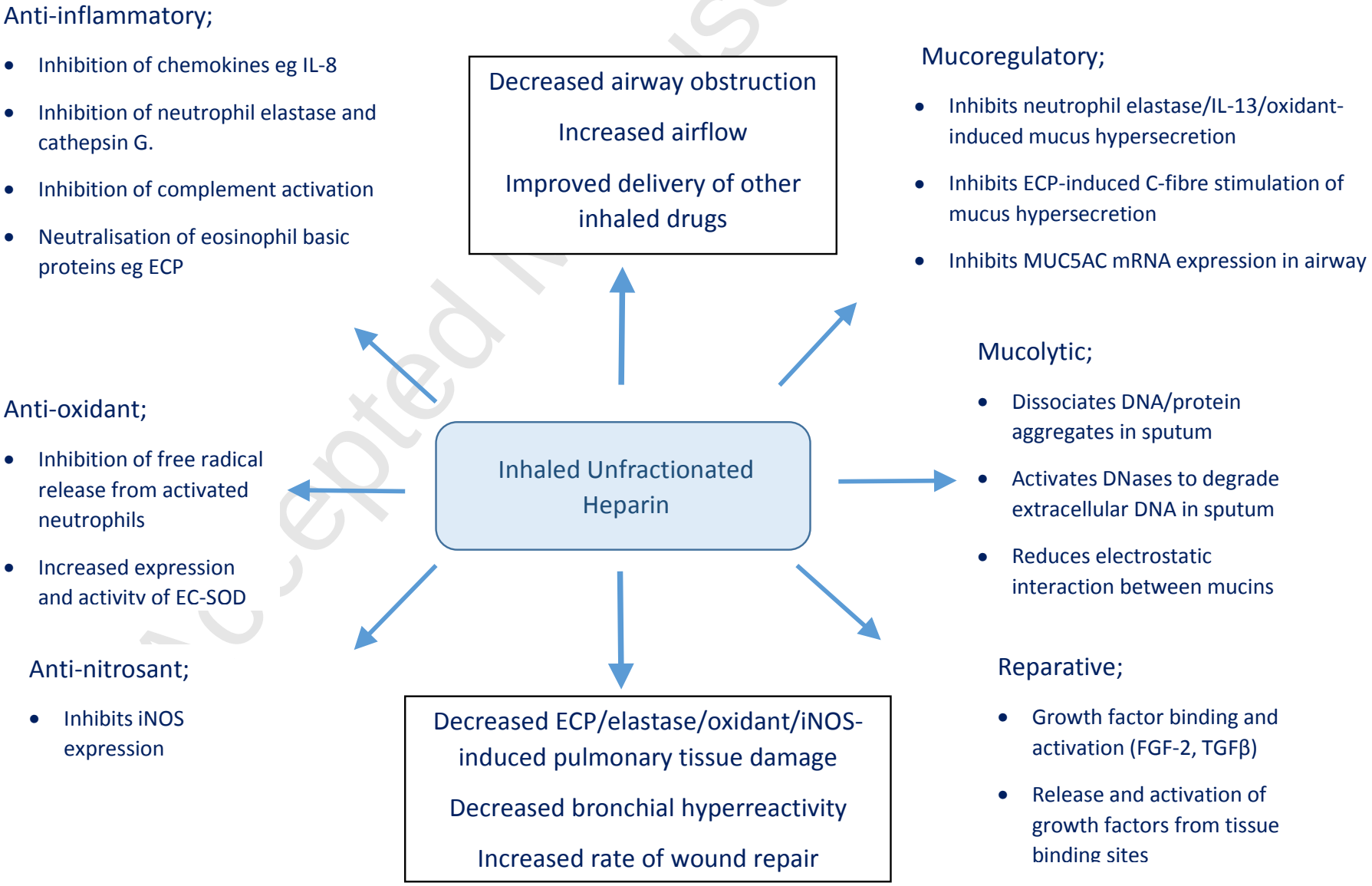

Figure 1. Potential mechanisms of action of inhaled unfractionated heparin in the treatment of asthma and COPD

Author (year)	Study design	Drug and dose	Patients	Clinical condition	Therapeutic outcome
Boyle et al. (1964). Am J Cardiol;14:25-8.	Double blind placebo (saline) controlled.	UFH - IV. 20,000 IU followed by 10,000 IU for 3 more doses every 12 h.	N=19 in test arm N=20 in control arm Equal numbers in groups A and B	A: Status asthmaticus or B: chronic bronchopulmonary disease with bronchospasm and tenacious secretions	Release of bronchospasm and elimination of obstructing mucus in 11 patients.
Youngchaiyud et al. (1969) Am Rev Resp Dis;99:449-452	Open label. Placebo (saline and heparin vehicle) controlled	**Inhaled**, nebulised UFH 20,000 IU in 1 ml 0.5% phenol -saline control.	N=20 in test arm N=17 in saline arm N=18 in 0.5% phenol vehicle control arm	COPD patients. FEV_1/FVC = 36-49%	Both heparin and the vehicle control induced expectoration. An expectorant action was not attributable to heparin. Heparin increased airway conductance, not seen in either control group.
Brown et al (2006) Pulm Pharmacol Ther;19:419-24.	Randomised parallel group. No control, treatment added to inhaled fixed dose salmeterol/fluticasone	LMWH enoxaparin, 20 mg – SC, once a day for 12 weeks	N=20 in control arm N=16 in treatment (completed)	Stable COPD FEV_1; Control mean 46.8% predicted, Test mean 47.8% predicted	Significant improvement in lung function by 4 weeks with enoxaparin, but in control, significant improvement only seen at 12 weeks.
Shi X & Li H (2013) Exp Ther Med;5:1367-1370	Randomised, open label	4100 IU LMWH-Ca (SC), bd, for 10 days	N=32 control, N=38 treated plus standard therapy	Acute exacerbation of COPD, on admission	Improved pulmonary function>control. Improved hypercoagulative state in treated group, not in control.
Qian et al (2014) COPD;11:171-6.	Randomised, open label.	LMWH nadroparin (SC) plus standard therapy for 7 days	N=33 control (standard therapy) N=33 test arm (standard therapy plus LMWH	COPD acute exacerbation, in mechanically ventilated patients	Plasma CRP, IL-6, and fibrinogen were significantly decreased in the LMWH group. LMWH significantly reduced the mean duration of mechanical ventilation, the length of ICU and hospital stay (14.3 days vs. 11.3 days; $p < 0.01$).
Shute et al (2018). Pulm Pharmacol Ther. 48: 88-96	Randomised, three parallel groups, double blind.	**Inhaled** UFH, 75,000 or 150,000 IU bd for 21 days. Aerosolised water control.	N= 9, placebo (water) N= 6, 75,000 IU N=9, 150,000 IU (completed)	COPD, post-exacerbation FEV_1%; Placebo; 50.45±3.12, 75,000;48.57±2.25, 150,000;41.53±2.51	No systemic anti-coagulation. Significant improvement in FEV_1 at 7 days with 150,000 IU. At 21 days significant effect on gas trapping at high dose. Improvement in exercise capacity and dyspnea with both doses.

Table 1; Studies of UFH and LMWH in patients with COPD.

UFH; unfractionated heparin, LMWH; low molecular weight heparin, IV; intravenous, SC; subcutaneous.