The antimicrobial effect of heparin on common respiratory pathogens

Nebulised heparin has been used successfully as a mucolytic agent in patients with chronic sputum production.\textsuperscript{1,2} Topical and systemic heparin is also used to treat burns, where it promotes analgesia and tissue perfusion, leading to improved healing and reduction in contractures.\textsuperscript{3–5} Heparin may also help prevent respiratory infections or minimise their severity through its mucolytic properties,\textsuperscript{1} combined with its anticoagulation, anti-inflammatory and neo-angiogenic characteristics.\textsuperscript{6–10} However, the antimicrobial contribution of heparin in respiratory infection has not been assessed. We examined the effect of unfractionated heparin on in-vitro growth of common respiratory pathogens.

Methods

Test organisms

Thirty individual isolates were tested, comprising isolates of \textit{Acinetobacter baumannii} (\(n = 4\)), \textit{Candida albicans} (\(n = 5\)), \textit{Haemophilus influenzae} (\(n = 5\)), \textit{Klebsiella pneumoniae} (\(n = 4\)), meticillin-resistant \textit{Staphylococcus aureus} (MRSA) (\(n = 3\)), \textit{Pseudomonas aeruginosa} (\(n = 2\)), and \textit{Streptococcus pneumoniae} (\(n = 7\)). The organisms were randomly selected from clinical isolates obtained from sputum, endotracheal aspirates and broncho-alveolar lavage specimens of patients in the intensive care unit at the Royal Brisbane and Women’s Hospital, Brisbane, QLD. These isolates had been stored on beads in glycerol at \(-70^\circ\text{C}\) in the Department of Microbiology, which routinely retains all clinically significant isolates indefinitely.

Preparation of microorganisms

Immediately after removal from the \(-70^\circ\text{C}\) freezer, the microorganisms were cultured on 5\% horse blood agar (bioMérieux, l’Etoile, France) for \textit{A. baumannii}, \textit{K. pneumoniae}, MRSA, \textit{P. aeruginosa} and \textit{S. pneumoniae}; chocolate agar (bioMérieux) for \textit{H. influenzae}; or Sabouraud agar (bioMérieux) for \textit{C. albicans}. Cultures were incubated overnight at 35\(^\circ\text{C}\) in an atmosphere with 5\% CO\(_2\). Isolated colonies were then subcultured to obtain individual pure colonies. A selection of isolated colonies of each organism was suspended in tryptic soy broth at a concentration of 1 McFarland standard (measured by nephelometer), with the exception of \textit{S. pneumoniae}, which was suspended in Todd Hewitt broth, also at a concentration of 1 McFarland.\textsuperscript{11}

Microbroth dilution method

All isolates were tested against heparin in sterile disposable plastic microtitre plates using the microbroth dilution method. To each well, we added 100\,\mu L of a heparin suspension containing 250–2500\,U unfractionated heparin, obtained from the commercially available porcine mucous heparin preparations supplied to our hospital (25\,000\,U/50\,mL [Baxter], 25\,000\,U/5\,mL [Pfizer], 5000\,U/1\,mL, 1000\,U/1\,mL, or 5000\,U/0.2\,mL [David Bull Labora-

ABSTRACT

Aim: The mucolytic, anticoagulative, anti-inflammatory and neo-angiogenic properties of inhaled heparin may benefit patients with burns and cystic fibrosis. We assessed the antibacterial effects of unfractionated heparin.

Methods: Stored clinical isolates of \textit{Acinetobacter baumannii} (\(n = 4\)), \textit{Candida albicans} (\(n = 5\)), \textit{Haemophilus influenzae} (\(n = 5\)), \textit{Klebsiella pneumoniae} (\(n = 4\)), meticillin-resistant \textit{Staphylococcus aureus} (MRSA) (\(n = 3\)), \textit{Pseudomonas aeruginosa} (\(n = 2\)), and \textit{Streptococcus pneumoniae} (\(n = 7\)) were subcultured on horse blood agar, incubated at 35\(^\circ\text{C}\) overnight, then inoculated into trypticase soy broth to a density of 1 McFarland standard. Dilutions of unfractionated heparin (containing 250–7500\,U) and 100\,\mu L of the 1.0 McFarland standard broth were incubated at 35\(^\circ\text{C}\) overnight in microtitre plates and then subcultured on horse blood agar using 1\,\mu L standard loops. Colonies (representing viable organisms) were counted.

Results: Heparin produced dose-dependent growth inhibition of three of seven \textit{S. pneumoniae} isolates (complete inhibition at 2500\,U dose per 200\,\mu L) and one of five \textit{H. influenzae} isolates (complete inhibition at 7500\,U dose per 200\,\mu L), but no inhibition of other isolates.

Conclusions: Unfractionated heparin is unlikely to have antibacterial effects because of its unpredictable inhibition of growth of common respiratory pathogens.

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These doses were chosen as they had been used in previously published clinical studies.5,12-16

To each well, we also added 100 μL of a suspension of freshly cultured test organism (concentration, 1 McFarland standard, as described above). Each microtitre plate also included positive controls (organism, no heparin) and negative controls (heparin, no organism). Inoculated trays were sealed with self-adhesive plastic sheets and incubated at 37°C in an atmosphere of 5% CO₂ for 24 hours. The optical density of each well was read using an optical reader, but minimum inhibitory concentration was difficult to interpret from visual turbidity.

Colony counts were determined by subculturing from each well using a standard 1 μL loop onto appropriate agar plates to allow determination of the minimum bactericidal concentration. Plates were incubated for 48 hours at 37°C before counting. Colony counts > 50 per plate were rounded to the nearest 10, while counts ≤50 were recorded as exact numbers of colony forming units. If an isolate showed evidence of growth inhibition at a heparin dose of 2500 U per 200 μL, then a fresh culture was tested against a second heparin preparation diluted to a concentration of 7500 U per 200 μL.

Results

The effects of heparin on growth of H. influenzae and S. pneumoniae are shown in Table 1 and Table 2, respectively. One H. influenzae isolate showed some growth inhibition at a heparin concentration of 2500 U per 200 μL, and complete growth inhibition at 7500 U heparin (Isolate 5).

Isolates 4, 5 and 6 of S. pneumoniae showed dose-dependent growth inhibition by heparin, with Isolates 5 and 6 completely inhibited at 7500 U per 200 μL. Isolate 7 grew poorly at all heparin concentrations as well as on the control plate, reflecting overall poor growth potential in vitro. Isolates 2 and 3 showed variable growth suppression compared with the control plate, and Isolate 1 was not inhibited at any of the heparin concentrations tested (Table 2).

No isolates of A. baumannii, C. albicans, K. pneumo-
niae, MRSA or P. aeruginosa showed any growth inhibition at heparin concentrations up to 7500 U per 200 μL.

Discussion

Heparin is a naturally occurring sulfated glycosaminoglycan that modulates the activity of numerous biological systems.2-4,7-9,17,18 Its anti-inflammatory and mucolytic effects may be helpful in treatment of respiratory infections.1,4,7-9,17-19 Our study showed a limited and variable antibacterial effect of unfractionated heparin on H. influenzae and S. pneumoniae isolates.

The methods used to detect bacterial growth inhibition were standard laboratory procedures, and the concentrations of heparin used related to existing clinical practice for nebulised heparin.20-23 The organisms chosen for this study reflected ICU respiratory pathogens. We noticed some isolates had dose-dependent growth inhibition with heparin, while others were resistant or had more variable growth patterns.

The mechanism of antibacterial action of heparin remains obscure. Heparin molecules are expressed within or on the surface of a number of tissues and promote adhesion with cells, extracellular matrix and growth factor proteins. Heparin is ubiquitously expressed in tissues with ability to bind a variety of bacteria and viruses.1,2,6,12 Heparin’s efficacy may stem from its highly sulfated, charged nature, which may alter interactions between charged molecules and chelation of key cations, interfere with hydrogen bonding or allow it to bind directly to organisms.1,2,5,6,12-16
Bacterial growth inhibition following central venous catheter locking with heparin has been attributed to chelation of divalent cations such as calcium and magnesium.\textsuperscript{14} Chelation of divalent cations can interfere with the integrity of bacterial cells through degradation of the cell wall membrane. Additionally, calcium may regulate several genes responsible for growth and survival of microbes.\textsuperscript{14} Depletion of calcium through chelation may also prevent formation of biofilms, which are presumed to have a role in infection related to artificial airways.\textsuperscript{5,14,15} Similarly, Pascu et al found that binding of \textit{S. aureus} to the heparin-binding growth factors, basic fibroblast growth factor and platelet-derived growth factor, is inhibited by heparin,\textsuperscript{16} which may thus impede infection. Similar interference with bacterial interaction at epithelial surfaces may explain the activity of heparin in our experiment.

In cystic fibrosis, both heparin and dextran can inhibit the infection-initiating step of bacterial adherence to mucous or epithelial receptors.\textsuperscript{13} Additionally, heparin thins sputum via charge interactions. In patients with cystic fibrosis and \textit{Burkholderia cepacia} infection, administration of nebulised heparin reduced both sputum and serum cytokine concentration, potentially aiding mucociliary clearance.\textsuperscript{1}

Nebulised heparin appears safe as it is metabolised by alveolar macrophages, capillaries, the endothelium of larger vessels, and the lining cells of lymphatics.\textsuperscript{21} It was found to be distributed uniformly throughout the lungs from which it cleared slowly. Less than 1% of the dose could be measured in blood following a nebulised dose of 90 000 U, far greater than the doses used in our study.\textsuperscript{12} In both asthma and cystic fibrosis, coagulation parameters remain normal with the doses of nebulised heparin described clinically.\textsuperscript{17,21,22} Massive doses of intrapulmonary heparin are required to prolong clotting time, but have not been associated with bleeding.\textsuperscript{21} In our study, the maximum heparin dose used was 7500 U, consistent with doses used in various clinical trials.\textsuperscript{1,8,16-18,23}

Conclusions

Unfractionated heparin produced variable growth inhibition of \textit{S. pneumoniae} and \textit{H. influenzae} isolates and had no effect on isolates of other organisms tested. It is therefore unreliable as an antibacterial agent. There are obvious clinical benefits from use of heparin therapy, and any additional impact on pulmonary infection does not depend entirely on its antibacterial effects. Conventional antibiotic therapy remains the mainstay for treatment of bacterial infections. Further studies are required to define any role for nebulised heparin in the prevention and treatment of respiratory disease.

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