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SCIENCE DIRECT
Pulmonary Pharmacology & Therapeutics 19 (2006) 286–291
PULMONARY PHARMACOLOGY & THERAPEUTICS
www.elsevier.com/locate/ypupt

Inhaled hyaluronic acid against exercise-induced bronchoconstriction in asthma

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Received 28 October 2004; revised 7 April 2005; accepted 27 April 2005

Abstract

Hyaluronic acid (HA) is a polysaccharide that is present in human tissues and body fluids. HA has various functions, including a barrier effect, water homeostasis, stabilizing the extracellular matrix, increased mucociliary clearance and elastin injury prevention. It may therefore exert prophylactic activity in the treatment of asthma. We tested the hypothesis that HA inhalation will prevent exercise-induced bronchoconstriction (EIB) in a randomised double-blinded placebo-controlled crossover study. Sixteen asthmatic patients with EIB were included in the study (mean (SD)) (age 24.5 (7.3) yr, FEV_1 88.6 (11.3) %predicted, PC_{20} methacholine (g-mean (SD in DD)) 0.4 (1.5) mg/ml). On two separate visits an exercise challenge was performed 15 min post-inhalation of either HA (3 ml 0.1% in PBS) or placebo (3 ml PBS). The maximum fall in FEV_1 and the AUC 30 min post-exercise were used as outcomes. After inhalation of both HA and placebo, baseline FEV_1 decreased significantly (HA 4.1 (3.1)%, placebo 2.9 (4.1)%, $P<0.017$). The maximum fall in FEV_1 following exercise challenge was not significantly different between HA versus placebo (median HA 22.50%, placebo 27.20%, $P=0.379$), as was the AUC (median HA 379.3 min*%fall, placebo 498.9 min*%fall, $P=0.501$). We conclude that at the current dose, inhaled HA does not significantly protect against EIB. This suggests that HA is not effective as a prophylaxis for EIB in patients with asthma.

Keywords: hyaluronic acid; asthma; exercise-induced bronchoconstriction; exercise challenge; treatment; randomized placebo-controlled study

1. Introduction

Hyaluronic acid (HA) is a naturally occurring polysaccharide, which can be found in numerous tissues and body fluids of vertebrates. HA can be found in high concentrations in the skin, synovial fluid, vitreous body and umbilical cord and in lesser amounts in the lung, kidney, brain, muscle and lymphatic fluid [1]. HA has several physiological functions and mechanisms, such as a barrier effect, water homeostasis, stabilizing the extracellular matrix, increased mucociliary clearance and elastin injury prevention [1–3].

Asthma is characterized by recurring periods of wheezing, chest tightness and coughing following (non)-specific triggers [4]. Exercise is a non-specific trigger, which can cause a short-lasting asthma attack that is often referred as 'Exercise-Induced Bronchoconstriction' (EIB). The asthmatic symptoms develop after approximately 8 min of strenuous exercise and peak at 8–15 min after cessation of the exercise. EIB is mediated by hyperventilation, which leads to heat and/or water loss from the airways. This causes changes in osmolarity and temperature of the bronchial mucosa, which can stimulate airway epithelial cells, infiltrative cells and airway nerves [5,6]. These pathways indirectly induce smooth muscle contraction and thereby EIB [5]. Through its barrier properties, HA may prevent heat and water loss from the airways during exercise and could thereby protect against EIB.

In sheep, HA blocks acute bronchoconstriction caused by human neutrophil elastase [7]. There are only limited data on protective activity of HA against bronchoconstriction in man. A first open-label trial in man with a single dose of inhaled HA was suggestive of a protective effect against EIB, both when using the fall in FEV_1 (Forced Expiratory Volume in one second) and PEF (Peak Expiratory Flow) as outcome in patients with asthma [8,9]. In patients with chronic obstructive pulmonary disease (COPD), 6 month treatment with HA resulted in fewer exacerbations and less

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1094-5539/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.pupt.2005.04.011

use of antibiotics, due to a reduction in the number of bacterial infections [10].

We hypothesized that inhaled HA protects against EIB in patients with asthma. The aim of this study was to test this hypothesis by examining the efficacy of inhaled HA compared with placebo against EIB in adults with asthma. As a secondary objective we evaluated the safety of inhaled HA by measuring alveolar gas exchange by carbon monoxide diffusion capacity pre- and post-treatment and monitored adverse events. To that end we performed a double-blind placebo-controlled crossover trial in asthmatic patients with EIB and compared the maximum fall in FEV_1 and the Area Under the time-response Curve (AUC) over 30 min post-exercise between HA and placebo treatment prior to an exercise challenge.

2. Methods

2.1. Patients

Sixteen non-smoking, atopic asthmatic patients with exercise-induced bronchoconstriction aged between 19 and 45 year were enrolled in this study (6 males, 10 females) [Table 1]. All included patients were atopic for one or more common air born allergens like house dust mite, grass, cat and/or dog. They had to fulfill the following inclusion criteria: clinical history of asthma, Forced Expiratory Volume in one second (FEV_1) $\geqq$75%predicted, concentration methacholine at which the patient had a fall in FEV_1 of 20% (PC_{20} methacholine) of $<$8 mg/ml, and $>$15% fall from baseline FEV_1 within 30 min after an exercise challenge. Only on-demand usage of short-acting β_2-agonists was allowed during the study, whereas inhaled corticosteroids and long-acting bronchodilators were discontinued for at least one month and two weeks, respectively, prior to the screening visit. At least two weeks prior to and during the study, none of the patients were having an upper respiratory infection or relevant allergen exposure. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center. Written informed consent was obtained from all patients according to the ICH Guidelines for Good Clinical Practices.

Table 1
Patient characteristics

	Mean (SD)
Gender (m:f)*	6:10
age (y)	24.5 (7.3)
Asthma duration (y)	18.5 (10.2)
pre-bronchodilator FEV_1 (l)	3.37 (0.61)
pre-bronchodilator FEV_1 (%predicted)	88.6 (11.3)
PC_{20} (mg/ml)#	0.4 (1.5)

*Number; #geometric mean (SD in doubling dose).

2.2. Design

The study had a randomized, double-blind, placebo-controlled cross-over design, which consisted of two screening visits and two treatment visits. At the first screening, a medical history was taken, spirometry was performed and PC_{20} methacholine was determined. At the second screening, an exercise challenge was performed. Blood and urine samples were taken as control measures.

At both treatment visits, baseline FEV_1 was determined prior to nebulization of the blinded medication. The patients inhaled either HA or placebo for 10–15 min. Directly thereafter post-treatment FEV_1 measurements were performed and the exercise challenge was started 15 min after ending of inhalation. The wash-out period between both treatment days was 7–14 days. The carbon monoxide diffusion capacity was measured both before treatment and after the exercise challenge in order to monitor a potentially adverse barrier effect of HA in the alveoli.

2.3. Methacholine challenge

The methacholine challenge was performed using the tidal breathing method described by the ERS [11]. A dry-rolling seal spirometer (Morgan, Spiroflow) was used for spirometry measurements. The diluent (saline 0.9%) was nebulized for 2 min using a jet-nebulizer (DeVilbiss, Somerset, PA, model 646, output 0.13 mg/min). The patient was wearing a nose-clip. Doubling doses of methacholine bromide (Janssen Pharmaceutica, Beerse, Belgium) between 0.15 μmol/ml – 40 μmol/ml were nebulized for 2 min at 5 min intervals. The FEV_1 response was determined at 30 and 90 seconds after nebulization. The test was discontinued if the FEV_1 dropped $\geqq$20%, if the highest concentration (40 μmol/ml) was reached or if the patient experienced serious discomfort. A dosage of 200 μg inhaled salbutamol was administered per pressurized Metered Dose Inhaler (pMDI) connected to an aerosol chamber in order to resolve bronchoconstriction after the test [11].

2.4. Exercise challenge

An exercise challenge was performed according to a standardized protocol [11], using a bicycle ergometer (Jaeger ER900). FEV_1 measurements were performed with a calibrated spirometer for a pre-exercise or baseline FEV_1 value (KoKo spirometer, PDS Instrumentation, Louisville, CO, US).

On the bicycle ergometer, the patient was wearing a nose clip and a facemask. The mask was connected to an air supply bag via a Hans Rudolph three-way valve. The patient inspired compressed dry air (20 °C, relative humidity $<$6% H_2O) and expired into the ambient air. The bicycle ergometer was started at a power of 20% of the predicted maximum power of the patient. The exercise intensity was increased until a minute ventilation of 40–50% of predicted