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LUNG SCINTIGRAPHY TO ASSESS THE ROLE OF LEVOSALBUTAMOL ON MUCOCILIARY CLEARANCE IN HEALTHY AND ASTHMATIC SUBJECTS

Clinical Research

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ABSTRACT

Objectives: We tested the effect of multi-dose levosalbutamol on bronchodilator responseand lung mucociliary clearance (MCC) in healthy and asthmatic subjectsfor its likely therapeutic role in asthma associated cough.

Material and Methods: An open label, single arm, multi-dose study was designedto assess bronchodilator response and MCC following administration of levosalbutamol (0.63 mg/2.5 mL) aerosol in 3 healthy and 3 asthmaticsubjects (FEV1 ≥80%, prescribed with inhaled corticosteroids), aged 29 to 45 years.

Results: Levosalbutamol treatment increases the FEV1 and FVC by 23.03 ±11.77% and 24.77±1.67%,respectively in asthmatics and by 4.67±1.88% and 6.03±6.79%, respectively in healthy subjects. Further, it also increases MCC from 43.7 ± 1.12% to 55.1 ± 11.16% inasthmatics and from 32.5 ± 13.87% to 62.2 ± 13.27% in healthy subjects by improving cilia beat frequency.

Conclusion: Levosalbutamol treatment demonstrated quick and persistent improvement in mucociliary clearance for likely incremental role in productive cough management in asthmatics and healthy.

KEYWORDS

Levosalbutamol; Asthma;Cough; Mucociliary clearance

INTRODUCTION

Lung mucociliary clearance (MCC) is the primary innate defense mechanism of the airways with the airway surface liquid layer, the protective mucous layer, and the cilia as the functional components (Nawroth et al., 2020). In pulmonarydisease such as asthma, the impaired MCC alongside airway inflammation results in cough associated with asthma (Bateman et al., 1983; Whitsett, 2018). It has been postulated thatduring an asthma episode, a cilia-inhibiting factordisorganizes ciliary beating and reducesciliary activity. Further, mucus hypersecretion, mucus retention and airway obstruction in asthmatics may also reduceciliary activity (Dulfano & Luk, 1983;Bonser et al., 2016). The increased quantity of viscous secretions is cleared less easily by a damaged ciliated epitheliumand therefore requires relief of cough symptoms alongside anti-asthmatic medications (Isawaet al., 1987; Yi et al., 2020).

Though mucolytics or bromhexine improve inhaled aerosol lung retention ratio their action is slow and inadequate(Davies &Webster, 1987). ACCP (Dicpinigaitis, 2006) and Cochrane review (Gadomski & Scribani, 2014) have suggested thetherapeutic roleof inhaled or oral bronchodilatorsin bronchial asthma associated cough. Beta-adrenergic agonists,primarily intended for relieving bronchospasm, may have an additional beneficial effects (Camner et al., 1976). The stimulatory effect of beta-adrenergics on MCC hasbeen coherently documented and various in vitro and in vivo animal studies reported favorable effectson mucus secretion (Phipps et al., 1980) and ciliary beat frequencies (Van, 1974; Verdugo et al., 1980). However, studies in healthy human volunteers and asthmatics are limited and inconclusive (Mossberg et al., 1976; Mortensen et al., 1993; Bennett, 2002). Levosalbutamol, an anti-asthmatic drug, is an important ingredient of cough formulations (Vora & Bhargava, 2016).Considering the possible benefit of levosalbutamol in bronchial asthma associated cough, we tested the effect of a short course of levosalbutamol inhalation on vital parameters, lung performanceand MCC in healthy and asthmatic subjects. The purpose of the present study was to investigatewhether improvement in MCCwould actually occurin patients withbronchial asthmawhen bronchodilation wasattained by inhaling levosalbutamol. In the present study, lung performance was measured by forced expiratory volume at 1 second (FEV1) and forced vital capacity (FVC) using aspirometer and MCC was assessed usingthe radiolabeled aerosol of technetium-99m diethylenetriamine penta-acetic acid (Tc-DTPA)(Karacavus & Intepe, 2015; Li, 2000).

MATERIALAND METHODS

Study Design

An open label, single-period, single center, single arm, multi dose inhalation study was designed to assess bronchodilator response and MCC following administration of levosalbutamol (0.63 mg/2.5 mL) aerosol in 3 healthy and 3 asthmaticsubjects.Two (02) healthy and one (01) asthmatic male and one (01) healthy and two (02) asthmatic females with mean age38 year old rangingfrom 29 to 45 were included in the study. All the subjects were non-smokers and gave informed consent in writing. The study was conducted at Nuclear Medicine Department, Molecular Imaging & TherapyDiagnostic Centre (Sanjay Nagar, Ghaziabad, UP) as per ICH-GCP guidelines followingIEC approval (GSER/2018/NR-AP/016; dated 05-09-2018) and under supervision of Nuclear Medicine specialist. Asthmatic subjects having mild to moderate asthma (FEV1 ≥80%), prescribed with inhaled corticosteroidswere included in the study.The therapeutic regimen of the asthmatics (primary treatment) was continued during the study.All the subjects were givenlevosalbutamol (0.63 mg/ 2.5 mL, Levolin Repsules, Cipla) using atomizer jet nebulizer (NE C28, Omron, Japan), t.d.s. every 6-8 hours for 3 days (total 9 doses). Subjects were screened for demographic data, medical and medication history and physical examination. Hematological tests, biochemistry, urine analysis, kidney function and liver function tests were conducted before commencement of the study and post study. Vital parameters, bronchodilator response and MCC was measured prior to administration of levosalbutamol (baseline) and immediately after the last dose (treatment).

Vital Parameters and Safety

Vital parameters such as weight (kg), height (cm), pulse rate (beats/min), body temperature (ºC) and blood pressure(mm Hg) were measured at baseline and post treatment. Subjects were also asked for any effect felt or observed during- and post-dosing to assess treatment effect.

Bronchodilator Response

To assess the effect of multi-dose levosalbutamol treatment on lung performance, bronchodilator responses such as forced expiratory volume at 1 second (FEV1) andforced vital capacity (FVC)were measured using aspirometer (Helios 702, RMS, India).

Mucociliary Clearance

Whole lung MCC was assessed usingthe radiolabeled aerosol of Tc-

DTPA as described previously (Karacavus & Intepe, 2015; Li, 2000). Subjects inhaled Tc-DTPA in acontrolled manner using nebulizer (NEC28, Omron, Japan). Inhalation volume was 2.5 mL containing 500-700 µCi of technetium pertechnetate. For quantitative analysis, the lung periphery wasoutlined on the computer display using thestatic-gamma image recorded at each time point andtwo regions of interest were then selected from each lung representing thecentral and peripheral regions. The distribution of deposited particles counts emanating from these regions were recorded immediately after radioaerosolinhalation and at 15 min, 30 min, 45 min, 60 min, 120 min, 240 min and 360 min using agamma camera. This procedure was carried out for both the left and the right lung and the results were averaged for each subject.MCC was calculated as percent count decay (initial count – count retained) at a particular time taking background andradioactive decay into consideration. The activity att = 0 (initial count) was considered as 100%retention. Central / peripheral (C/P) ratio was calculated by dividing radioactive counts of central region by peripheral region.

Statistical Analysis

All data is presented as the mean ±SD. Data for vital parameters and MCC was analyzed with Student's t test andP valueless than 0.05 was considered statistically significant. For bronchodilator response the changes were reported as percentage and the statistical significance was not applied due to small sample size (n=3) and deviation in the baseline values.

RESULTS

Vital Parameters and Safety

Following levosalbutamol treatment no significant change (P > 0.05) in weight, height, pulse rate, body temperature and blood pressure was observed in either healthy or asthmatic subjects (Table 1). Two asthmatics reported improvement in breathing post treatment. Whilst, two healthy subjects reported dizziness and heavy head post treatment which lasted for about 20 to 30 minutes.

Table 1. Vital parameters of subjects at baseline and post levosalbutamol treatment

Parameters	Healthy Subject		Asthmatic Subject	
	Baseline	Post-treatment	Baseline	Post-treatment
Weight (Kg)	68.7 ± 3.1	68.7 ± 3.1	68.0 ± 3.6	68.0 ± 3.6
Height (cm)	169.7 ± 3.5	169.7 ± 3.5	165.1 ± 6.8	165.1 ± 6.8
Pulse rate	80.7 ± 6.4	78.0 ± 7.2	80.7 ± 5.8	80.3 ± 4.0
Body temperature (ºC)	36.9 ± 0.2	37.2 ± 0.2	37.1 ± 0.4	37.2 ± 0.4
Diastolic blood pressure (mm Hg)	86.7 ± 2.9	83.3 ± 5.8	89.7 ± 2.5	85.0 ± 5.0
Systolic blood pressure (mm Hg)	122.7 ± 4.6	123.3 ± 5.8	136.0 ± 5.3	133.3 ± 2.9

Bronchodilator Response

Table 2 shows individual FEV1 and FVC values of healthy and asthmatic subjects at baseline and post levosalbutamol treatment. As expected, the baseline FEV1 (2.96 ± 0.39) and FVC (3.37±0.66) values of healthy subjects were 50.3% and 49.8% higher than baseline FEV1 (1.47 ± 0.63) and FVC (1.69 ± 0.62) values of asthmatics, respectively. Levosalbutamol treatment resulted in marked bronchodilatation in all the 3 asthmatic subjects and the mean change in FEV1 and FVC was 23.03 ±11.77 % and 24.77±1.67 %, respectively. In healthy subjects, following levosalbutamol treatment, FEV1 and FVC was increased by 4.67±1.88 % and 6.03±6.79 %, respectively.

Table 2. Bronchodilator response of subjects at baseline and post levosalbutamol treatment

PFT Values	FEV1			FVC		
	Baseline	Post-treatment	% Decrease	Baseline	Post-treatment	% Decrease
Healthy Subjects						
1	2.55	2.72	6.7%	2.62	2.97	13.4%
2	3.32	3.42	3.0%	3.85	3.83	0.0%
3	3.02	3.15	4.3%	3.65	3.84	5.2%
Mean	2.96±0.39	3.09±0.35	4.67±1.88	3.37±0.66	3.55±0.50	6.03±6.79
Asthmatic Subjects						
1	1.22	1.41	15.6%	1.22	1.51	23.8%
2	2.19	2.56	16.9%	2.39	2.96	23.8%
3	1.01	1.38	36.6%	1.46	1.85	26.7%
Mean	1.47±0.63	1.78±0.67	23.03±11.77	1.69±0.62	2.10±0.75	24.77±1.67

Mucociliary Clearance

The representative lung deposition pattern of the Tc-DTPA at different time points in the healthy and asthmatic subject is shown in Figure 1. Baseline scintigraphic images showed widespread deposition of Tc-DTPAin central and peripheral regions of the lungs (Data not shown) with no significantdifference in C/P ratios (P>0.05) betweenhealthy (1.49) and asthmatics (1.54). A statistically significant change from 1.49 to 1.24 in healthy (P<0.05)and from 1.54 to 1.25 in asthmatics (P<0.05)was observed after levosalbutamol treatment. However, the post-treatmentC/P ratiosbetweenhealthy (1.24) and asthmatics (1.25)were not significantly different (P>0.05).

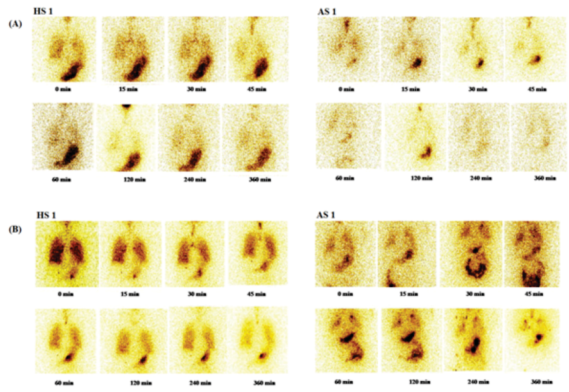

Figure 1. The representative lung deposition pattern of the Tc-DTPA at different time points in the healthy (HS1) and asthmatic (AS1) subject at (A) baseline and (B) post-levosalbutamol treatment.

The MCC ofradioactiveTc-DTPA at baseline and post-levosalbutamol treatment in healthy and asthmatic subjects is shown in Figure 2(A) and 2(B), respectively.Data comparison was done at 6 h time point. At baseline, MCC was significantly higherfor asthmatic subjects (43.7 ± 1.12%) compared to healthy (32.5 ± 13.87%). Levosalbutamol treatment increases MCC from 43.7 ± 1.12% to 55.1 ± 11.16% inasthmatics and from 32.5 ± 13.87% to 62.2 ± 13.27% in healthy subjects. In contrast to bronchodilator responses, the increase in MCC was more pronounced in healthy subjects compared to asthmatics.

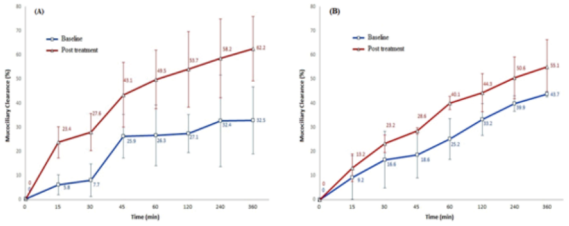

Figure 2. Mucociliary clearance ofTc-DTPA at baseline and post-levosalbutamol treatment in (A) healthy subjects and (B) asthmatic subjects

DISCUSSION

Considering the probable benefit of levosalbutamol in both asthma and cough, we tested the effect of short course levosalbutamol on vital parameters, bronchodilator responseand MCC in healthy and asthmatic subjects.Cough regimen was followed and levosalbutamol nebulization was given three times daily every 6 to 8 hours for 3 days.The baseline estimations of the same subject prior to levosalbutamol treatment was considered as control.The use of baseline estimations of the same subject minimizes data variability. To perceive clinically realistic results,both healthy and asthmatics subjects were enrolled and primaryasthmatic medication of the asthmatics was continued throughout levosalbutamol treatment period. To our knowledge this is the first report where lung performance of β-agonist was evaluated along-side administration of primary asthma medication.

As expected, following levosalbutamol treatment, the vital parameters

such as weight, height, pulse rate, body temperatureand blood pressure remain unchanged in both healthy and asthmatics. Breathing improvement in 2/3 asthmaticswas possibly due to bronchodilation by levosalbutamol. Common, mild side-effects of levosalbutamol such as transient dizziness and heavy headwere reported for short duration by 2/3 healthy subjects. Overall the treatment was well tolerated.

Asthmatics have inflamed airways and bronchospasm(Holgate, 2008), which lead to increased airway resistance and thus the baseline FEV1 and FVC values of asthmatics were significantly lower than those ofhealthy subjects.Levosalbutamol treatment resulted in enhanced bronchodilator response in both healthy and asthmatic subjects.It is well known that β-agonists relax muscles of the airway, widen the airways and improves lung performance (Vora & Bhargava, 2016). The increase in FEV1 and FVC was found to be more pronounced in asthmatics compared to healthy subjects,which is consistent with previous observationswhereβ-agonistsdemonstrated significantly improved lung performancein asthmatics compared to healthy subjects (Bateman et al., 1983a; Bennett, 2002).

Scintigraphy has been reported to demonstrate the MCC assuming that radiolabel marker depositing on the airway surface moves out of the lung at the same rate as the airway secretions in which it is immersed (Li, 2000). We have used 99mTc-DTPAaerosol particlesdue to itsease of preparation, ease of availability and low cost. Based on results of previous studies (Bateman et al., 1983a), it was expected that single dose levosalbutamol would not affect MCC and thus multi-dose studies was planned.The estimation of C/P helps to explicate that the change in MCC, if observed, is due to improvement in ciliary beat frequency or due to changes in deposition(Shah et al., 2006). In asthmatics, the inhaled Tc-DTPA would be expected to deposit more centrally. This presumption stems from studies of induced bronchoconstriction where deposition shifts to more central airways(Clague et al., 1983). Contrastively, in our study we found that the C/P for healthy and asthmatics did not differ significantly.This could possibly be due to bronchodilation caused by primary medication in asthmatics. Levosalbutamol treatment resulted in shift of deposition to peripheral airwaysand a decrease in C/P ratio in both healthy and asthmatics. These observation are clear indicative of bronchodilationcaused by levosalbutamol. MCC is often decreased in patients with asthma or COPD due to impaired mucociliary transport particularly in peripheral airways, which may arise due to impaired cilia function (Whitsett, 2018). Aikawa et al (1989) also observed that mucus retention occurred especially in the peripheral airways. In our studies, baseline MCC, six hours after radioaerosol inhalation,werehigher for asthmatics compared to healthysubjects. The contradictory results could be explained considering three assumptions: (a) in previous reports primary asthma medication was discontinued prior to commencement of study (Bateman et al., 1983), (b) more central deposition of radioaerosol as demonstrated by C/P of 1.54, and (c) one healthy subject demonstrating very low MCC value resulting in lowering of mean MCC and large standard deviation.

The levosalbutamol treatment enhances MCC in both asthmatic and healthy subjects. From the results it is clearly evident that levosalbutamol treatment improves ciliary beat frequency in asthmatics and healthy subjects. Data of C/P study showed that levosalbutamol treatment resulted in shift of deposition to peripheral airways which are characterized by slow MCC (Shah et al., 2006). Thus, increase in MCC could be attributed to improvement in cilia function.

Also, the increase in MCCwas more pronounced in healthy compared to asthmatics possibly due to better lung condition as depicted by higher FEV1 and FVC values. Several studies demonstrated enhancement of whole lung clearance by β-agonist in healthy subjects, however results in patients with the airway diseases are less convincing(Bennett, 2002). To date, no study has demonstrated an enhancement of mucus clearance from levosalbutamol inhalation. In our study, enhancement of MCC was observed in both healthy and asthmatics possibly due to multi-dose, short-term levosalbutamol treatment andcontinuation of primary medication with levosalbutamol treatment. Though the results were diluted for background inhaler therapy with corticosteroids, the lack of side effects augers well for wider acceptance of bronchodilator based cough formulations for hypersecretory or chronic obstructive airway diseases including asthma, cystic fibrosis and tuberculosis that has wider representation and implication in developing countries like India.

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