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Research Article

PHARMACOKINETIC DOSE-DECELERATION STUDY IN EVIDENCE-BASED MEDICAL PHARMACOVIGILANCE EVALUATION OF ANTI-ASTHMATIC AEROSOLISED LEVOSALBUTAMOL, AND QUALITATIVE PHARMACOGENOMIC ASSOCIATION ANALYSIS BETWEEN ADRB2 GENETIC POLYMORPHISMS AND β2SYMPATHOMIMETIC MECHANISMS: A MIXED METHOD CLINICAL RESEARCH

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ABSTRACT

Introduction: Levosalbutamol is the purified enantiomer of mixed dextro and levo-rotatory racemic salbutamol, that has a quite high affinity for the β2 receptor. Occupation of β2 receptors by levosalbutamol causes the activation of the Gs-adenylyl cyclase-cAMP-PKA pathway, resulting in phosphorylative events, leading to bronchial smooth muscle relaxation.
Objectives: The objective of this mixed method clinical research was a pharmacokinetic dose-deceleration studyin the evidence-based medical pharmacovigilance evaluation of anti-asthmatic aerosolised levosalbutamol, and qualitative pharmacogenomic association analysis between *ADRB2* genetic single nucleotide polymorphisms and β2 sympathomimetic mechanisms.
Methods: 52 global patients, with mild to early moderate asthma, were prescribed the inhalation treatment of levosalbutamol 50mcg per actuation, with a metered dose inhaler, 2 puffs in each nostril, once in the early morning, and once in the early evening, for 1 month, and then, 2 puffs in each nostril, once in the early evening, for the next 1.5 months. After each levosalbutamol inhalation dose, the patients were monitored for 24 hours, for the occurrence of any adverse effect, like headache, tremor, irritation in the oral cavity and palpitation, with Adverse Event Case Report Forms, on 0, 3, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 84, 98, 112, and further follow-ups. The patient findings were recorded and statistically analysed. A qualitative pharmacogenomic analysis of *ADRB2* genetic single nucleotide polymorphisms association with β2 sympathomimetic mechanisms, was also conducted, with global epigenetic considerations.
Results: There were no occurrence of any adverse drug reaction with 50 mcg levosalbutamol (2 puffs in each nostril BD) and 50 mcg levosalbutamol (2 puffs in each nostril OD) inhalation treatment. The adverse effects of levosalbutamol were not statistically significant; and the stepwise dose decrease of aerosol inhalation of levosalbutamol, were equally safe and tolerable. The pharmacogenomic analysis showed a reasonably substantial association between *ADRB2* genetic polymorphisms and β2 sympathomimetic mechanisms.
Conclusions: This safety evaluation study concluded thatanti-asthmatic levosalbutamol aerosol inhalation was safe and tolerable, during the stepwise dose deceleration, with substantial association with *ADRB2* genetic polymorphisms.

INTRODUCTION

Asthma is a chronic inflammatory disease of the airways, characterized by increased bronchial hyperresponsiveness, bronchoconstriction, microvascular leakage and plasma exudation, due to activation of M3 receptors on bronchial smooth muscle by increased cGMP levels, on release of acetylcholine, caused by released histamine, leukotriene C4, D4, B4, prostaglandin D2, protease enzymes, TNFα, platelet activating factor, interleukins (IL-4, IL-5, IL-13), adenosine, eosinophil cationic protein, neuropeptides (substance-P and neurokinin-A), from the allergen induced activated mast cells,

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infiltrating eosinophils, basophils and T helper 2 lymphocytes.[1] Short-acting β2 agonists, levosalbutamol, is one of the the most commonly used bronchodilators for the routine treatment of mild to early moderate asthma.

Levosalbutamol is the purified (R, R) enantiomer of mixed dextro and levo-rotatory racemic diastereomer (R, R / S, S) salbutamol, that has a quite high affinity for the β2 receptor. Occupation of β_2 receptors by levosalbutamol causes the activation of the Gs-adenylyl cyclase-cAMP-PKA pathway, resulting in phosphorylative events, leading to bronchial smooth muscle relaxation.By this pharmacodynamic mechanism, levosalbutamol causes bronchodilatation, inhibition of inflammation and baseline airway reversibility.[1،2]

Aerosol inhalation of levosalbutamol is administered to the asthmatic patients by the portable and the very convenient metered dose inhaler, a drug delivery system, conventionally used in the treatment of mild to early moderate asthma.

A metered dose inhaler is a topical drug delivery system, conventionally used in the treatment of mild asthma. It is portable, very convenient, cost-effective, less time consuming, less energy resources consuming, requiring less maintenance and with lesser adverse effects like oro-respiratory mucosal irritation.

Inhaled medications for asthma are available as pressurised metered dose inhaler, metered dose inhaler with spacer, breath-actuated metered dose inhaler, dry powder inhalers, soft mist inhalers and nebulised or wet aerosols.

Inhaler devices differ in their efficacy of drug delivery to the lower respiratory tract, depending on:

1. form of devices
2. formulation of medication
3. particle size
4. velocity of aerosol cloud or plume
5. ease with which device can be used by majority of patients

Studies have shown no statistically significant differences on spirometric variables like peak expiratory flow rate (PEFR), forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC) and FEV1/FVC, after giving bronchodilators, by metered dose inhaler, nebuliser or dry powder inhaler.

Choice of an inhaler device depends on

1. patient's age
2. cognitive status
3. visual acuity
4. manual dexterity and strength
5. ability to coordinate inhaler actuation with inhalation
6. disease severity
7. convenient to use
8. portability
9. cost-effectiveness.[3]

Objectives

The objective of this mixed method clinical research was a pharmacokinetic dose-deceleration study in the evidence-based medical pharmacovigilance evaluation of anti-asthmatic aerosolised levosalbutamol, and qualitative pharmacogenomic association analysis between *ADRB2* genetic single nucleotide polymorphisms and β_2 sympathomimetic mechanisms.

METHODS

Study design

The study type was a prospective, multi-centre, open-labelleddose-variation pharmacokinetic and pharmacovigilance study of safety and tolerability evaluation, with dose deceleration; and aqualitative pharmacogenomic analysis of *ADRB2* genetic polymorphisms association with β_2 sympathomimetic mechanisms.

Study population

The study population was 52global patients, suffering from mild to early moderate asthma.

Place of Study

The place of study, including the research study and the compilation of the study literature were the Departments of Pharmacology, Clinical Pharmacology, Pharmacovigilance, Rational Pharmacotherapeutics, Molecular Pharmacology, Evidence-Based Medicine, Pharmacogenomics, Medical Genetics, Clinical Pathology, Pathology, Molecular Diagnostics, Respiratory Medicine, and Clinical Medicine, Mamata Medical College and Hospitals, Rama Medical College Hospital and Research Centre, Rama University, Hazra Nursing Home, Dr. Moumita Hazra's Polyclinic And Diagnostic Centre, Dr. Moumita Hazra's Academic Centre, Dr. Moumita Hazra's Educational Centre, Gouri Devi Institute of Medical Sciences and Hospital, and J. J. M. Medical College and Hospitals.

Study period

The study period was 5 months: from December, 2012 to April, 2012; June, 2021, and December, 2021.

Ethical approval

At first, the clearance and the approval from the Institutional Ethics Committee were obtained. The study was conducted in accordance with the ethical principles originating from the Declaration of Helsinki and Good Clinical Practices contained within the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH-E6 and ICH-E17), and in compliance with the global regulatory requirements. Informed consent was obtained from each patient.

Selective Criteria of the Study Participants

Inclusion criteria: were as following:

1. Age > 18 years, of any gender;
2. British Thoracic Society definition of asthma grades;[4]
3. Ability to perform spirometry maneuvers;
4. Co-operative and conscious patients.

Exclusion criteria: were as following

1. Uncooperative and unconscious patients;
2. Patients presenting with acute severe or acute life-threatening or near-fatal asthma;
3. History of hypersensitivity to the study drugs;
4. Pregnant or lactating women;
5. Other associated medical illness having impact on study results;
6. Children or very old patients.

Study Procedure

52global patients, with mild to early moderate asthma, were selected for this research study. After obtaining the clearance from the Institutional Ethics Committee and informed consent, the following data of the thorough patients' history with complete examination details and prescription patterns were obtained with the study proforma : the patients' participation assessment and adherence to treatment (including patients who completed the study thoroughly, number of drop-out patients to adverse effects, patients who were lost to follow-up and patients who withdrew voluntarily); the demographic characteristics, including age, gender, race, body mass index, duration of symptoms of asthma, severity of asthma symptoms, present controller medications, the patients' present and past history, smoking history, respiratory history including respiratory immunological history and history of allergy, chronic obstructive pulmonary disease and asthma, cardiac history, history of co-morbidities, family history, personal history, socio-economic history, reproductive history, concomitant medication history, and surgical history were recorded. The Saint George's Respiratory Questionnaire (SGRQ) scores, and the Baseline Dyspnea Index (BDI) / Transition Dyspnea Index (TDI) questionnaire scores, were recorded, to assess the effect of treatment on asthma.[5] Details of complete general physical examination, and systemic examination, including oto-rhino-laryngo-tracheal, respiratory and cardio-pulmonary examinations, were recorded. Pulse rate, oxygen saturation of arterial haemoglobin (SpO2) and respiratory rate were recorded with a Peak Flow Meter.

Spirometric variables like peak expiratory flow rate (PEFR), forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC) and FEV1/FVC, were recorded, after giving bronchodilators, by metered dose inhaler. The patients were prescribed the inhalation treatment of levosalbutamol 50mcg per actuation, with a metered dose inhaler, 2 puffs in each nostril, once in the early morning, and once in the early evening, for 1 month, and then, 2 puffs in each nostril, once in the early evening, for the next 1.5 months. After each levosalbutamol inhalation dose, the patients were monitored for 24 hours, for the occurrence of any adverse effect, like headache, tremor, irritation in the oral cavity and palpitation, with Adverse Event Case Report Forms, on 0, 3, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 84, 98, 112, and further follow-ups.

A qualitative pharmacogenomic analysis of *ADRB2* genetic single nucleotide polymorphisms association with β2 sympathomimeticmechanisms was conducted, wherein, the extensive and varied pharmacogenomic study literature, was also thoroughly analysed from wide ranged molecular pharmacological research, review and case presentations, keeping in consideration the global epigenetic manifestations.

Statistical analysis

The study findings were statistically analysed, with tabular illustrations, for the decelerated dose-variations, along with the test of significance, being denoted by the p-value (p-value ≤ 0.05: statistically significant), and subsequent tabular illustrations.

RESULTS AND DISCUSSION

In this study, there were no occurrence of any adverse drug reaction with 50 mcg levosalbutamol (2 puffs in each nostril BD) and 50 mcg levosalbutamol (2 puffs in each nostril OD) inhalation treatment, as depicted in Table 1 and Table 2. The adverse effects of levosalbutamol were not statistically significant; and the stepwise dose decrease of aerosol inhalation of levosalbutamol, were equally safe and tolerable. The demographic characteristics were comparable.

Table 1 Adverse drug reactions of 50 mcg levosalbutamol (2 puffs in each nostril BD) and their frequency

Sl. no.	**Adverse drug reactions of 50 mcg levosalbutamol**2 puffs in each nostril BD	**Number of patient occurrence of levosalbutamol** (n=52)	p-value
1.	Headache	0	ns
2.	Tremor	0	ns
3.	Irritation in oral cavity	0	ns
4.	Palpitations	0	ns

BD = Twice a day, ns = Non-significant

Table 2 Adverse drug reactions of 50 mcg levosalbutamol (2 puffs in each nostril OD) and their frequency

Sl. no.	**Adverse drug reactions of 50 mcg levosalbutamol** 2 puffs in each nostril OD	**Number of patient occurrence of levosalbutamol** (n=52)	p-value
1.	Headache	0	ns
2.	Tremor	0	ns
3.	Irritation in oral cavity	0	ns
4.	Palpitations	0	ns

OD = Once a day, ns = Non-significant

All the patients completed the treatment thoroughly. There were no drop-out patients due to adverse effects, none was lost to follow-up and none of the patients withdrew voluntarily. The patients' adherence to treatment was very high.

The metered dose inhaler was a very convenient drug delivery medical device for the administration of levosalbutamol, as an aerosolised inhalation, among all age-groups of mild to early moderate asthmatic patients.

This qualitative pharmacogenomic analysis of *ADRB2* genetic single nucleotide polymorphisms association with β2 sympathomimetic mechanisms, along with the analyses of the global epigenetic manifestations, showed that there is amoderately substantial association between non-synonymous single nucleotide polymorphisms (SNPs) of *ADRB2*gene at codons 16 and 27 and β2sympathomimetic mechanisms.

Asthma is a complex cardio-respiratory disease, caused by the unique interactions between genetic and environmental factors. Identification of the genetic basis of asthma may contribute to the development of innovative anti-asthmatic drugs. Nocturnal asthma represents a subset of asthma, who usually experience worsening symptoms, and airflow obstruction caused due to bronchoconstriction, during the night. Nocturnal asthma is associated with significant decline in pulmonary function and increase in airway inflammation at night. Nocturnal asthma is a severe form of asthma, and it is associated with increased morbidity, which has an extreme negative impact on the quality of life for patients. Patients with nocturnal asthma were generally found to have >15% decrease in lung function during the night. A number of single nucleotide polymorphisms in the β2-AR gene, namely Arg/Gly at codon 16 and Gln/Glu at codon 27, have been implicated in asthma susceptibility. A single amino acid substitution in the structural domains critical for receptor function has been shown to result in significant changes in receptor function.

The gene for the human beta2-adrenergic receptor (β2-AR) is located on chromosome5q31. The most frequent single

nucleotide polymorphisms (SNPs) in the β2-AR gene are due to two missense mutations, which occur in the coding region of the intronless β2-AR gene. The first SNP (A > G) at nucleotide 46 causes the substitution of glycine (Gly) for arginine (Arg) at codon 16. The second SNP (C > G) at nucleotide 79 results in the substitution of glutamic (Glu) acid for glutamine (Gln) at codon 27.

Some studies have indicated that Gly16 allele is associated with enhanced agonist-mediated downregulation of the receptor, while Glu27 allele enhances resistance to downregulation.[6 - 8]

Many studies have stated that there are ethnic variations in the prevalence of the two β2-AR SNPs. These polymorphisms, which cause downregulation of the β2-AR, play a significant role in bronchial asthma since they are linked with functional changes in the β2-AR in the respiratory system.

Certain studies found no difference in allele frequency of these two polymorphisms in patients with and without asthma.

While, few studies have shown that there is increased risk of asthma susceptibility in patients with these polymorphisms.

A meta-analytical study concluded that neither polymorphism influences the risk for asthma.

Quite some studies had been performed to determinewhether these polymorphisms influence morespecific asthma phenotypes, such as the severeform of the disease or nocturnal asthma.

With β-agonists being the most widely used agents in the treatment of asthma, *in vitro* studies reported that β-adrenergic receptor (*ADRB2*) polymorphisms are associated with agonist-promoted down-regulation. Non-synonymous single nucleotide polymorphisms (SNPs) of *ADRB2* at codons 16 and 27 are quite significant.

In a study, the β2-AR gene polymorphisms at codon 16 and 27 were assessed in 40 patients, who were clinically diagnosed with nocturnal asthma and 96 normal controls. Genomic DNA was obtained from whole blood and genotyping was carried out by a PCR based restriction fragment length polymorphism technique. Statistical analysis Fisher's Exact test and Hardy–Weinberg equilibrium (HWE) for the control group and linkage disequilibrium (LD), haplotypes frequencies estimation, and haplotypes association analysis with SNP Stats web tool for association studies were performed. It was found that there was a statistically significant difference in genotype frequencies at codon 16 (Arg/Gly) betweennocturnal asthmatic patients and normal control subjects ($P < 0.05$). However, there was no statistically significant difference in allele frequencies between the two groups. In addition, there was a significant association between Arg16-Gly genotype with nocturnal asthma compared to homozygous Gly16 (codominant model P = 0.0033, OR = 3.69: 95% CI: 1.49–9.12). Although, there were no statistically significant differences in genotype and allele frequencies at codon 27 (Gln/Glu) between the normal control and nocturnal asthmatic groups ($\chi 2 = 1.81$, P = 0.41). The results also indicate that linkage disequilibrium existed between the β2-AR codon 16 and β2-AR codon 27 polymorphism ($|D'| = 0.577$). The data for all haplotypes did not show a statistically significant association. This study showed that there was a significant difference in the genotype and allele frequencies of β2-AR gene polymorphisms in normal subjects and nocturnal asthmatic patients, mostly in genotype frequencies at codon 16 (Arg/Gly). But, there was a poor association of individual single nucleotide polymorphisms with nocturnal asthma.[6]

In a recent meta-analytical study, the cumulative evidence of the effects of the association of nonsynonymous single nucleotide polymorphisms (Arg16Gly and Gln27Glu) in the adrenoceptor β2 (*ADRB2*) gene with percent forced expiratory volume in 1 second (FEV 1.0%) after β2 sympathomimetic bronchodilator use in asthmatics, showed that there were no statistically significant mean difference of FEV1.0% between genotypes of Arg16Gly and Gln27Glu. In the subgroup analyses, significant associations were found for Arg16Gly GG (*vs* AA) among studies where no methacholine bronchoconstriction was conducted (mean difference, -3.92; 95% confidence interval, -7.29 to -0.54; I^2= 0%), and for Arg16Gly GG (*vs* GA) among studies that included patients with no comorbidities (mean difference, -1.93; 95% confidence interval, -3.77 to -0.10; $I^2 = 0\%$). Therefore, a weak evidence was found for the association between *ADRB2* Arg 16Gly and Gln27Glu and FEV1.0%after β2 sympathomimetic bronchodilator use in asthmatics, with a significant underscoring of the heterogeneity among studies.[9]

Therefore, the preceding pharmacogenomic study literature corroborated a moderately substantial association between non-synonymous single nucleotide polymorphisms (SNPs) of *ADRB2* gene at codons 16 and 27 and β2 sympathomimetic mechanisms.

CONCLUSION

Inhaler administered stepwise dose decrease of levosalbutamol, in the aerosolised form, were equally safe, as the prevalent drug-through-the-device therapeutic system, for treating mild to early moderate asthma, with reasonably substantial association between *ADRB2* genetic polymorphisms and β2 sympathomimetic mechanisms.

This study would remain an essential juncture in the development of more efficacious and safe anti-asthmatic pharmacotherapeutic drugs, which would certainly retain, in better consideration, the pharmacogenomic and epigeneticrespiratory associations.

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References

1. Hazra M. A pharmacoepidemiological study of prescription patterns of β_2 sympathomimetic bronchodilators in exacerbation of non-severe asthma in tertiary care hospitals, not needing hospitalization. *Int. J. Basic Clin. Pharmacol*., 2019; 8: 2674-2680.
2. Hazra M., Geetha M., Somashekar H. S., Arun B. J. and Vidyasagar B. A study comparing rescue medications: arformoterol and salbutamol nebulization in exacerbation of non-severe asthma at a tertiary care center, not needing hospitalization. *Int. J. Pharm. Sci. Res*., 2013; 4(8): 3151-3156.
3. Rizvi DA, Abidi A, Agarwal A andAhmad A. Comparison of the efficacy of budesonide by nebuliser, metered dose inhaler and dry powder inhaler in chronic stable bronchial asthma. *Int. J. Basic Clin. Pharmacol*., 2018; 7: 1333-8.
4. British Thoracic Society and Scottish Inter Collegiate Guidelines Network. British Guidelines on the Management of Asthma. Thorax, 2008; 63(Suppl 4): 1-121.
5. Hanania N. A., Sethi S., Koltun A., Ward J. K., Spanton J. and Ng D. Long-term safety and efficacy of formoterol fumarate inhalation solution in patients with moderate-to-severe COPD. *Int. J. Chron. Obstruct. Pulmon. Dis*., 2019; 14: 117-27.
6. Al-Rubaish A. M. Association of beta2-adrenergic receptor gene polymorphisms and nocturnal asthma in Saudi patients. Ann. Thorac. Med., 2011; 6: 66-9.
7. Hall I. P. Beta2-adrenoceptor polymorphisms and asthma. Clin. Exp. Allerg.,1999; 29: 1151-4.
8. Hall I.P. Genetics and pulmonary medicine: Asthma. Thorax, 1999; 54: 65-9.
9. Hikino K., Kobayashi S., Ota E., Mushiroda T., Urayama K. Y. and Kobayashi T. A meta-analysis of the influence of *ADRB2* genetic polymorphisms on albuterol (salbutamol) therapy in patients with asthma. Br. J. Clin. Pharmacol., 2021; 87(4): 1708-1716.

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