**Name of Journal: *World Journal of Respirology***

**ESPS Manuscript NO: 20109**

**Manuscript Type: MINIREVIEWS**

**β2-adrenoceptor in obstructive airway diseases: Agonism, antagonism or both?**

Tan DWS *et al.* β2-adrenoceptor in obstructive airway diseases

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**Author contributions:** All authors equally contributed to the writing of the review article.

**Supported by** NMRC/CBRG/0027/2012 from the National Medical Research Council of Singapore (in part); and by NUHS Seed Fund R-184-000-238-112.

**Conflict-of-interest statement:** There is no conflict of interest

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**Received:** May 28, 2015

**Peer-review started:** May 28, 2015

**First decision:** August 4, 2015

**Revised:** September 1, 2015

**Accepted:** October 1, 2015

**Article in press:**

**Published online:**

**Abstract**

Obstructive airway disease is a complex disease entity including several maladies characterized by bronchoconstriction and abnormal airway inflammation. Reversing bronchoconstriction leads to symptomatic relief and improvement in quality of life, both in reversible (bronchial asthma) and partially reversible (chronic obstructive airway disease) obstructive airway diseases. β2-adrenoceptor expressed in human airway is the main β-receptor subtype, and its activation in airway smooth muscle cells leads to bronchodilatation. Drugs targeting β-adrenoceptors have been around for many years, for which agonists of the receptors are used in bronchodilation while antagonists are used in cardiovascular diseases. This review article summarizes the effect and usage of β2-agonist in obstructive airway disease, addressing the benefits and potential risks of β2-agonist. The article also looks at the safety of β-blocker usage for cardiovascular disease in patients with obstructive airway disease. There is also emerging evidence that non-selective β-blockers with inverse agonism ironically can have long-term beneficial effects in obstructive airway disease that is beyond cardiovascular protection. Further trials are urgently needed in this area as it might lead to a dramatic turnaround in clinical practice for obstructive airway diseases as has already been seen in the usage of β-blockers for heart failure.

**Key words:** β-adrenoceptors; β2-agonist; β-blocker; Inverse agonist; Heart failure; β-arrestin

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**Core tip:** This review summarizes the effect and usage of β2-agonist in obstructive airway disease, addressing the benefits and potential risks of β2-agonist. The review also looks at the safety of β-blocker for cardiovascular disease in patients with obstructive airway disease. There is also emerging evidence that non-selective β-blockers with inverse agonism ironically can have long-term beneficial effects in obstructive airway disease beyond cardiovascular protection. Further trials are urgently needed in this field as it might lead to a dramatic turnaround in clinical practice for obstructive airway diseases as has already been seen in the usage of β-blockers for heart failure.

Tan DWS, Wong JL, Tie ST, Abisheganaden JA, Lim AYH, Wong WSF. β2-adrenoceptor in obstructive airway diseases: Agonism, antagonism or both? *World J Respirol* 2015; In press

**INTRODUCTION**

According to the 2015 reports from Global initiative for Asthma (GINA) and Global initiative for chronic obstructive lung disease (GOLD), the prevalence of asthma ranges from 1% to 18% worldwide, while prevalence of chronic obstructive pulmonary disease (COPD) is about 6%[1,2]. Obstructive airway diseases, both asthma and COPD, are characterized by abnormal inflammation and bronchoconstriction. Bronchospasm is contributed by both airway smooth muscle contraction and mucus production by the epithelial cells. Pathogenesis of obstructive airway disease is therefore a complex interaction among inflammatory cells, epithelial cells of the bronchial airway, smooth muscle cells and fibroblasts. While the role of inflammation is emphasized in the pathogenesis and treatment of airway diseases, especially asthma, the role of airway smooth muscle cells beyond inflammation has been gaining increased recognition. This has led to the development of new β2-agonists, especially the long-acting β2-agonists since the 1990s. Their introduction into clinical practice however has generated some controversy. Recently, there was a paradigm shift in the understanding of obstructive airway disease and increasing evidence points to the role of β-blockers, especially those with inverse agonist action (or negative intrinsic efficacy), in the management of obstructive airway diseases.

***Adrenoceptors in the airways***

Adrenoceptors (AR) belong to the G protein-coupled receptor (GPCR) family and are activated by endogenous hormone adrenaline and neurotransmitter noradrenaline. Receptor activation stimulates the heterotrimeric G proteins (Gα and Gβγ subunits) and, in turn, the Gα subunit activates effector molecule (*e.g.*, adenylyl cyclase, phospholipase Cβ, and transducin) for signal transduction. Various subtypes of Gα protein have been described, including Gαq, Gαt, Gαs and Gαi proteins.

There are two main groups of AR which have been classified as α- and β-subtypes, and are encoded by at least nine unique genes (α1A,α1B,α1D,α2A/D, α2B, α2C, β1, β2 andβ3)[3]. α1-AR typically induce vascular smooth muscle contraction *via* a Gαq protein. α2-AR are mainly expressed in presynaptic terminals and regulate release of neurotransmitters. Despite evidence for α-AR distribution in the lung, neither receptor subtype has a clear role in regulating human airway smooth muscle tone or plays a significant role in the pathogenesis of asthma or COPD[4]. In contrast, β-AR activate adenylyl cylase *via* the Gαs protein to produce cyclic adenosine monophosphate (cAMP), which promotes airway smooth muscle relaxation (Figure 1).

β-AR are subdivided into at least three distinct groups: β1, β2, and β3. In mouse or guinea pig trachea, airway bronchial tissues have twice the density of β2-AR compared to β1, and the density of β3 is much less[5]. In humans, however, quantitative autoradiographic analyses of human isolated bronchus have shown that β-AR of airway smooth muscle are entirely of the β2-receptor subtype. Similarly, β-AR of airway epithelium are also entirely of the β2-receptor subtype. Only in bronchial sub-mucosal glands was β1-AR found[6]. As such, β2-AR play a more important role than β1-AR in the pathogenesis of obstructive airway diseases.

***Role of β2-AR in obstructive airway disease***

Studies using non-selective β-blockers with inverse agonism or β2-AR-/- knockout mice demonstrated that β2-AR signaling is required for the full asthma phenotypic development in mice[7].

Smooth muscle relaxation in the airways is one of the most critical targets of drug therapy during acute exacerbation of bronchial asthma. It is believed that β2 agonist action is primarily mediated by cAMP-dependent protein kinase A (PKA). Activated PKA will phosphorylate myosin light chain kinase, reducing its ability to activate myosin light chain which is essential for airway smooth muscle contraction, hence, leading to the bronchodilatory effect[3]. Another biologically important action of β2-AR agonist is to induce membrane hyperpolarization *via* activation of the K+ channels in the plasma membrane by PKA, which counteracts the electrical excitation and subsequent Ca2+ influx contributing to contraction[8]. Cyclic AMP has also been shown to cross-talk with the mitogen-activated protein kinase (MAPK) pathway through the inhibition of Ras-dependent activation of Raf, resulting in inhibition of this proliferative pathway. β2-agonist usage may prevent smooth muscle remodeling as well as contraction[9].

β2-AR are also found on the surface of bronchial epithelial cells. A study in transgenic mice shows that an over-expression of β2-AR on the epithelial cells of bronchial airway could prevent bronchoconstriction and hyperresponsiveness to methacholine. β-AR activation could lead to increase ciliary beat frequency and increase alveolar fluid clearance in animal and human lung tissues. β2-AR appear to be responsible for most of the β-receptor-sensitive alveolar active Na+ transport which facilitates alveolar fluid removal[10]. Experimental data also suggest that β2-agonist inhibits endothelial cell contraction and reduces intercellular gap, improving the endothelial barrier function. Human β2-AR have been shown to regulate mucin production and increase mucous viscosity. In animals, usage of β2-agonist is associated with increasing goblet cell hyperplasia[11], while the treatment with β-blockers in mouse epithelial cells significantly reduces the density of mucus-producing goblet cells[12].

The role of β2-AR in inflammatory cells is more controversial. *In vitro* studies of long-acting β2-agonists (LABA) formoterol and salmeterol show that activation of β2 receptors inhibited neutrophil and eosinophil adhesion to tracheal venules, and IL-1 and leukotriene B4 secretion from human alveolar macrophages[13]. β2-receptor activation inhibits the production of IL-6, IL-8, RANTES, eotaxin, GM-CSF, and MCP-1. However, some recent evidence has pointed towards the detrimental effects of LABA in promoting further inflammation in asthma. Loza *et al*[14] showed that β2-agonist promoted IL-13+ T-helper 2 cell survival by activation of the PKA pathway. An *in vitro* study by Oehme *et al*[15] demonstrated that prolonged treatment with β2-agonists reduced β2-receptor expression and stimulated IL-6 and IL-8 production in human bronchial epithelial cell line.

***β2-agonist and its role in obstructive airway disease***

The Chinese have been inhaling herbs containing ephedrine for asthma from centuries ago. In 1698, John Foyer[16] understood that asthma treatment is “both in fit and out of it”, suggesting early recognition of both acute treatment and maintenance therapy. Since the early 1900s, direct adrenergic bronchodilators were introduced in Western medicine for the treatment of asthmatic attacks[17], way before the usage of corticosteroids in the 1940s. During the 1960s and 1970s, relatively specific β2- agonists were developed for inhalational use[18]. The introduction of LABA such as salmeterol and formoterol in the 1990s was considered a major advancement in asthma therapy with evidence of improved lung function and quality of life. In 2011, the once daily β2-agonist indacaterol is being used in COPD patients[19].

Drugs that act on β2-AR are classified by their speed of onset, duration of action, affinity, intrinsic efficacy and potency. The duration of action and onset of action is influenced by lipophilicity and kinetics of binding. Among the agents currently used, salmeterol and formoterol sustain longer duration of action than salbutamol as their lipophilicity produces a depot effect at the cell membrane, allowing slow and sustained release of the drugs[20]. Formoterol has a shortened lipophilic side chain compared to salmeterol and hence while it’s moderate lipophilicity allows it to enter and be retained in the plasmalemma, sufficient drugs are still available in the aqueous biophase to allow immediate interaction with the active site of the receptor, accounting for its rapid onset of action.

The affinity of a drug depends on its specific binding to the β2-AR and is usually described in terms of dissociation constant between the agonist and the receptors. The intrinsic efficacy of a β2-AR agonist will depend on the ability of the drug to activate its receptor. Drugs that have high intrinsic efficacy are termed full agonist while drugs with lower intrinsic efficacy are termed partial agonist. The potency of a drug depends on both its affinity and intrinsic efficacy. Drugs that inhibit the β-AR (β-blockers) are either antagonists or inverse agonists. Antagonists are drugs that prevent the agonist from binding to the receptors, while inverse agonists are drugs that bind the receptor and inactivate constitutive downstream signaling. Many β-blockers in the market possess inverse agonist action on the β-AR, such as propranolol and nadolol, where they are able to inhibit constitutively active receptors[7].

Although the role of the β2-adrenergic agonists had long been recognized, their long term usage has been controversial. Occasional epidemics of asthma-related deaths have been linked to the use of β2-agonists such as fenoterol[21]. The Serevent National Survey (SNS)[22] study in the United Kingdom and the Salmeterol Multicenter Asthma Research Trial (SMART)[23] study in the United States raised the concern that regular usage of LABAs such as salmeterol may increase asthma-related mortality. This mortality is not seen when a LABA is used concomitantly with an inhaled corticosteroid[24]. The increased mortality is attributed to increased bronchial hyperresponsiveness, loss of protection against bronchoconstrictor stimuli and the development of tolerance[25].

It has long been appreciated that the ability of β2-agonist to induce bronchodilatation weans over time[26]. This is termed as loss of Broncho-Protective Effect (BPE) of β2-agonist, which was initially attributed to desensitization and down-regulation of the β2-AR (Figure 1). The mechanism for desensitization and down-regulation of β2-AR is linked to receptor phosphorylation by PKA and by β-adrenergic receptor kinase (βARK), a member of the G-protein receptor kinases (GRK), leading to conformational change in the receptor and its consequent reduced coupling to G proteins, leading to desensitization[27,28]. βARK also promotes the binding of β-arrestin proteins to the receptor[29]. Arrestins act as scaffolding proteins that allow desensitized receptors to undergo endocytosis into the cells, lysis, and termination of further signaling process.

***β2-blocker or inverse agonist and their role in obstructive airway disease***

Traditionally β-blockers have been contraindicated in various diseases including obstructive airways disease and congestive cardiac failure. A recently published study by Bellocchia *et al*[30], which recruited 229 patients, showed that 51% COPD and 30% asthmatic patients had cardiovascular disease. Congestive heart failure (CHF) in COPD patients range from 8% to 27% while coronary artery disease (CAD) in COPD patients range from 15% to 25%[31]. In a recent RHYTHMOS study, in a population of 280 CAD with COPD patients, only 52.8% were treated with β-blockers, where most were treated with sub-optimal dosages[32]. In another study by Puente-Maestu *et al*[31], only 58% of COPD patients with indication for CHF/CAD were prescribed with β-blockers, while 97% of non-COPD patients with indications were treated with β-blockers. Studies of using β-blockers in asthma and COPD have demonstrated decreased airway reversibility[33] and reduction in FEV1[34,35]. A large retrospective electronic medical record database review of 11592 adult patients with asthma and COPD by Brooks and co-workers in 2007 revealed that patients with asthma with or without COPD who were taking selective or non-selective β-blockers had an increased risk of hospitalization and emergency department visits[36]. All these added to the reluctance to use β-blockers in obstructive airway disease.

However, a recent single center randomized double-blind placebo-controlled trial with a sample size of 16 in the United Kingdom showed that 80 mg/d of propranolol given to patients with persistent asthma did not cause adverse effects[37,38]. Using an OVA-induced murine asthma model, nadolol, a non-selective β-blocker with inverse agonist action, was shown to reduce mucous metaplasia, BALF cellular infiltrates and airway hyperresponsiveness[7]. In a 4-mo rat model of smoking, it was shown that cigarette smoking leads to excessive sympathetic stimulation, resulting in down-regulation of β2-AR[39]. Propranolol was found to be able to reduce inflammatory cell infiltration in lungs, mucus secretion, TNF-α and IL-8 levels[40]. It also reduced norepinephrine level in the serum and increased airway smooth muscle response to isoprenaline[41]. These studies highlight the feasibility of using β-blockers in obstructive airway disease (Figure 2).

It has been shown that β-inverse agonists such as propranolol inhibit G protein-dependent signaling, but activate MAPK through β-arrestin in mouse embryonic fibroblasts and CHO cells[42,43]. β2-AR have been studied intensively, and depending on the ligand binding site, it can induce differential stabilized conformation which in turn elicits a variety of selectivity toward G-protein-dependent and β-arrestin-dependent signaling[44,45]. It was further proposed that a secondary binding site may be exposed upon adequate conformational state, leading to a different signaling cascade[44]. However, a recent study reveals that chronic propranolol treatment reduced MAPK activation through β-arrestin-dependent signaling, leading to reduced MUC5AC expression and mucus hypersecretion induced by cigarette smoke[46]. The discrepancy could be due to a different models with acute *vs* chronic treatment with propranolol. It has been reported that acute treatment with nadolol led to an increase in airway resistance to methacholine in a murine asthma model, but chronic administration reduced it together with lower mucin content[47]. In addition, chronic treatment with nadolol in HEK293 cells led to reduced β2-AR degradation and increased protein levels[47]. Therefore the beneficial effects of chronic treatment with β-inverse agonists are worthy of further investigation (Figure 3).

***Use of β-blocker for cardiovascular protective effects***

In 1975, Fynn Waagstein *et al*[48] published the first positive results using a β-blocker to treat congestive cardiac failure (CHF), and this led to the FDA in approving the usage of β-blockers in CHF. Since then, β-blockers have been widely used in treating patients with ischemic heart disease (IHD) and impaired cardiac contractility. However, a significant proportion of patients with IHD also have risk factors for COPD. Reluctance on usage of β-blockers in patients with COPD and asthma has become a major cause of under usage of β-blockers in IHD. In one study, COPD patients had a nearly two-fold increase in cardiovascular disease (CVD) death rates compared to the general population[49]. In fact, impaired lung function seems to be an independent risk factor for arrhythmias, coronary events, and all-cause mortality[50]. Therefore, it seems crucial to explore the potential survival benefit of using β-blockers in obstructive airways disease.

A meta-analysis by Salpeter *et al*[51] (2005) examined all randomized, blinded and controlled trials from 1966 to 2005, on the effect of single dose or longer duration cardio-selective β-blockers on FEV1 or symptoms in patients of COPD. This meta-analysis demonstrated that cardio-selective β-blockers do not affect the FEV1 or respiratory symptoms compared to placebo. It is also a relief to see that the cardio-selective β-blockers do not blunt the effect of β2-agonists on FEV1[51]. Another recent meta-analysis of observational studies also concluded that non-selective β-blockers can reduce overall mortality risk and exacerbation risk[52]. Over the past decade, the are a plethora of observational trials suggesting that non-selective β-blockers in patients with COPD is not only safe but beneficial in terms of reducing mortality, hospitalization, health-care utilization, and even admissions for respiratory disease including COPD exacerbations[31,34,53-55]. The benefit was not only shown in a wide range of COPD patients with CVD like hypertension, acute myocardial infarction[56,57], congestive heart failure and patients that underwent major vascular surgery[58], but it was also shown in patients without any overt cardiovascular disease[59]. Recent heart failure guidelines published by the Heart Failure Society of America recommend that for the majority of patient with left ventricular systolic dysfunction, cardioselective β-blocker therapy is recommended even in the presence of concomitant COPD[60]. Nevertheless, caution must be exercised as the non-selective β-blockers were associated with an increase rate of hospitalization and emergency room visits in the study by Brook *et al*[36].

***β-blockers beyond cardiovascular protective effects – the new frontier in asthma treatment***

There is good evidence to suggest at least the usage of cardio-selective β-blockers in patients with obstructive airway disease with concomitant CVD. However their role beyond cardiovascular protection is still unknown, especially in asthma. Since the publication of the SMART and SNS studies documenting the potential side effects of β2-agonist, several studies have now been undertaken to evaluate the role of chronic β-blocker usage in reducing the long term side effects of β2-agonist, and in asthma control beyond the cardiovascular protection. This is a very bold and exciting development in the field of asthma pharmacotherapy and control.

The safety of β-blockers has also been demonstrated in asthmatic patients. A recent observational study in Scotland investigated the effect of non-selective β-blockers in 1527 asthmatic patients. The study did not find any significant increase in steroid rescue use in β-blocker treatment group[61]. Another meta-analysis study of randomized, blinded, and placebo-controlled trials reveals that acute single dosing with cardioselective β-blockers produced a slight but significant reduction in FEV1 of 7.46% without affecting symptoms, while chronic dosing did not significantly reduce FEV1. In addition, a significant increase in subsequent β2-agonist response was seen upon chronic dosing, indicating that β2-receptor up-regulation might have occurred[34].

In an experimental asthma model, acute administration of β2-agonist salbutamol or alprenolol, a β-blocker without inverse agonist action, reduced airway resistance in mice, but upon chronic use, either drug did not affect the airway resistance response to antigen challenge. On the other hand, acute administration of β-AR inverse agonist nadolol or carvedilol did not affect airway responsiveness, but after 28 d of treatment, the inverse agonists markedly reduced airway responsiveness to antigen[62]. The beneficial effect may be contributed by an up-regulation β2-AR expression in chronic usage of the β-inverse agonist, as demonstrated by the increased receptor staining in histological lung sections[63]. Furthermore, chronic β-blocker usage also reduces eosinophilic inflammation, cytokine production, and mucin content in a chronic mouse asthma model[12].

These findings in murine models led to the first proof-of-concept open-label study by Hanania and co-workers. Ten patients with mild steroid-naive asthma (mean FEV1 of 90%) were given incremental doses of nadolol from 10 to 40 mg for 9 wk. There was an initial decrease in FEV1, but with chronic dosing this effect tended to ameliorate, and airway hyper-responsiveness to methacholine challenge significantly improved (amounting to 1.8 doubling doses in PC20, the provocative dose of methacholine that leads to a 20% fall in FEV1)[64]. The effect of another β-blocker propranolol was further tested in a randomized control trial conducted by Short *et al*[65]. Although the primary outcome of the trial was not met, the trial demonstrated the safety of β-blocker in carefully selected steroid-treated stable patients with asthma. The usage of concomitant inhaled steroid may have caused the up-regulation of β2-AR hence reducing the effect of the β-blocker[65]. More trials are warranted in this exciting field.

**CONCLUSION**

It is projected that obstructive airway disease will become the third leading cause of death by the year 2020 by the World Health Organization. Obstructive airway disease is a spectrum of disease that ranges from reversible bronchial asthma to irreversible COPD with significant overlap. Both inflammatory cells and resident cells expressing β2-AR are vital in the pathogenesis of obstructive airway disease. Anti-inflammatory drugs and β2-agonists are the pillars of treatment for the disease. Acute usage of β2-agonist allows bronchodilatation and symptomatic relief. However the long term use of LABA monotherapy has been linked to reduced bronchoprotective effect of the drugs.

Emerging evidence shows that β2-blockers, particularly those with inverse agonist action and cardio-selective properties are safe in obstructive airway disease and should be used for its cardioprotective effect in at-risk patients. There is also evidence of benefit beyond the cardioprotective effects, particularly in reversible airway disease[64]. The risk of β2-AR blockade-mediated bronchoconstriction should be balanced against the long-term benefit of β-blocker usage in asthma. While early clinical studies of β-blocker in asthma shows exciting and promising results, further larger, more comprehensive studies are needed to address both the safety and long term benefit of β-blocker before changes to the treatment of obstructive airway disease can be justified.

**REFERENCES**

1 **Global initiative for asthma**. Global strategy asthma management and prevention. 2015. Available from: URL: http://www.ginasthma.org

2 **Global initiative for chronic obstructive lung disease**. Global Strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. 2015. Available from: URL: http://www.goldcopd.org

3 **Giembycz MA**, Newton R. Beyond the dogma: novel beta2-adrenoceptor signalling in the airways. *Eur Respir J* 2006; **27**: 1286-1306 [PMID: 16772391 DOI: 10.1183/09031936.06.00112605]

4 **Goldie RG**. Receptors in asthmatic airways. *Am Rev Respir Dis* 1990; **141**: S151-S156 [PMID: 2155564 DOI: 10.1164/ajrccm/141.3\_Pt\_2.S151]

5 **Henry PJ**, Rigby PJ, Goldie RG. Distribution of beta 1- and beta 2-adrenoceptors in mouse trachea and lung: a quantitative autoradiographic study. *Br J Pharmacol* 1990; **99**: 136-144 [PMID: 1970491 DOI: 10.1111/j.1476-5381.1990.tb14667.x]

6 **Carstairs JR**, Nimmo AJ, Barnes PJ. Autoradiographic visualization of beta-adrenoceptor subtypes in human lung. *Am Rev Respir Dis* 1985; **132**: 541-547 [PMID: 2864008]

7 **Nguyen LP**, Lin R, Parra S, Omoluabi O, Hanania NA, Tuvim MJ, Knoll BJ, Dickey BF, Bond RA. Beta2-adrenoceptor signaling is required for the development of an asthma phenotype in a murine model. *Proc Natl Acad Sci U S A* 2009; **106**: 2435-2440 [PMID: 19171883 DOI: 10.1073/pnas.0810902106]

8 **Billington CK**, Ojo OO, Penn RB, Ito S. cAMP regulation of airway smooth muscle function. *Pulm Pharmacol Ther* 2013; **26**: 112-120 [PMID: 22634112 DOI: 10.1016/j.pupt.2012.05.007]

9 **Cook SJ**, McCormick F. Inhibition by cAMP of Ras-dependent activation of Raf. *Science* 1993; **262**: 1069-1072 [PMID: 7694367]

10 **Mutlu GM**, Dumasius V, Burhop J, McShane PJ, Meng FJ, Welch L, Dumasius A, Mohebahmadi N, Thakuria G, Hardiman K, Matalon S, Hollenberg S, Factor P. Upregulation of alveolar epithelial active Na+ transport is dependent on beta2-adrenergic receptor signaling. *Circ Res* 2004; **94**: 1091-1100 [PMID: 15016730 DOI: 10.1161/01.RES.0000125623.56442.20]

11 **Kamachi A**, Munakata M, Nasuhara Y, Nishimura M, Ohtsuka Y, Amishima M, Takahashi T, Homma Y, Kawakami Y. Enhancement of goblet cell hyperplasia and airway hyperresponsiveness by salbutamol in a rat model of atopic asthma. *Thorax* 2001; **56**: 19-24 [PMID: 11120899 DOI: 10.1136/thorax.56.1.19]

12 **Nguyen LP**, Omoluabi O, Parra S, Frieske JM, Clement C, Ammar-Aouchiche Z, Ho SB, Ehre C, Kesimer M, Knoll BJ, Tuvim MJ, Dickey BF, Bond RA. Chronic exposure to beta-blockers attenuates inflammation and mucin content in a murine asthma model. *Am J Respir Cell Mol Biol* 2008; **38**: 256-262 [PMID: 18096872 DOI: 10.1165/rcmb.2007-0279RC]

13 **Johnson M**. Effects of beta2-agonists on resident and infiltrating inflammatory cells. *J Allergy Clin Immunol* 2002; **110**: S282-S290 [PMID: 12464937 DOI: 10.1067/mai.2002.129430]

14 **Loza MJ**, Peters SP, Foster S, Khan IU, Penn RB. beta-Agonist enhances type 2 T-cell survival and accumulation. *J Allergy Clin Immunol* 2007; **119**: 235-244 [PMID: 17208607 DOI: 10.1016/j.jaci.2006.09.019]

15 **Oehme S**, Mittag A, Schrödl W, Tarnok A, Nieber K, Abraham G. Agonist-induced β2-adrenoceptor desensitization and downregulation enhance pro-inflammatory cytokine release in human bronchial epithelial cells. *Pulm Pharmacol Ther* 2015; **30**: 110-120 [PMID: 24915152 DOI: 10.1016/j.pupt.2014.05.007]

16 **Floyer JS**. A treatise of the asthma. London: R. Wilkins, 1698

17 **Melland B**. The treatment of spasmodic asthma by the hypodermic injection of adrenalin. *Lancet* 1910; **175**: 1407–1411 [DOI: 10.1016/S0140-6736(01)14446-6]

18 **Smith JM**. The recent history of the treatment of asthma: a personal view. *Thorax* 1983; **38**: 244-253 [PMID: 6408749]

19 **Mahler DA**, D'Urzo A, Bateman ED, Ozkan SA, White T, Peckitt C, Lassen C, Kramer B. Concurrent use of indacaterol plus tiotropium in patients with COPD provides superior bronchodilation compared with tiotropium alone: a randomised, double-blind comparison. *Thorax* 2012; **67**: 781-788 [PMID: 22544891]

20 **Hanania NA**, Dickey BF, Bond RA. Clinical implications of the intrinsic efficacy of beta-adrenoceptor drugs in asthma: full, partial and inverse agonism. *Curr Opin Pulm Med* 2010; **16**: 1-5 [PMID: 19887938]

21 **Pearce N**, Beasley R, Crane J, Burgess C, Jackson R. End of the New Zealand asthma mortality epidemic. *Lancet* 1995; **345**: 41-44 [PMID: 7799709]

22 **Castle W**, Fuller R, Hall J, Palmer J. Serevent nationwide surveillance study: comparison of salmeterol with salbutamol in asthmatic patients who require regular bronchodilator treatment. *BMJ* 1993; **306**: 1034-1037 [PMID: 8098238]

23 **Nelson HS**, Weiss ST, Bleecker ER, Yancey SW, Dorinsky PM. The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. *Chest* 2006; **129**: 15-26 [PMID: 16424409 DOI: 10.1378/chest.129.1.15]

24 **Jaeschke R**, O'Byrne PM, Mejza F, Nair P, Lesniak W, Brozek J, Thabane L, Cheng J, Schünemann HJ, Sears MR, Guyatt G. The safety of long-acting beta-agonists among patients with asthma using inhaled corticosteroids: systematic review and metaanalysis. *Am J Respir Crit Care Med* 2008; **178**: 1009-1016 [PMID: 18776152 DOI: 10.1164/rccm.200804-494OC]

25 **Lin R**, Degan S, Theriot BS, Fischer BM, Strachan RT, Liang J, Pierce RA, Sunday ME, Noble PW, Kraft M, Brody AR, Walker JK. Chronic treatment in vivo with β-adrenoceptor agonists induces dysfunction of airway β(2) -adrenoceptors and exacerbates lung inflammation in mice. *Br J Pharmacol* 2012; **165**: 2365-2377 [PMID: 22013997 DOI: 10.1111/j.1476-5381.2011.01725.x]

26 **Larj MJ**, Bleecker ER. Effects of beta2-agonists on airway tone and bronchial responsiveness. *J Allergy Clin Immunol* 2002; **110**: S304-S312 [PMID: 12464940 DOI: 10.1067/mai.2002.130045]

27 **Morgan SJ**, Deshpande DA, Tiegs BC, Misior AM, Yan H, Hershfeld AV, Rich TC, Panettieri RA, An SS, Penn RB. β-Agonist-mediated relaxation of airway smooth muscle is protein kinase A-dependent. *J Biol Chem* 2014; **289**: 23065-23074 [PMID: 24973219 DOI: 10.1074/jbc.M114.557652]

28 **Benovic JL**, Pike LJ, Cerione RA, Staniszewski C, Yoshimasa T, Codina J, Caron MG, Lefkowitz RJ. Phosphorylation of the mammalian beta-adrenergic receptor by cyclic AMP-dependent protein kinase. Regulation of the rate of receptor phosphorylation and dephosphorylation by agonist occupancy and effects on coupling of the receptor to the stimulatory guanine nucleotide regulatory protein. *J Biol Chem* 1985; **260**: 7094-7101 [PMID: 2987243]

29 **Penn RB**, Pascual RM, Kim YM, Mundell SJ, Krymskaya VP, Panettieri RA, Benovic JL. Arrestin specificity for G protein-coupled receptors in human airway smooth muscle. *J Biol Chem* 2001; **276**: 32648-32656 [PMID: 11418617 DOI: 10.1074/jbc.M104143200]

30 **Bellocchia M**, Masoero M, Ciuffreda A, Croce S, Vaudano A, Torchio R, Boita M, Bucca C. Predictors of cardiovascular disease in asthma and chronic obstructive pulmonary disease. *Multidiscip Respir Med* 2013; **8**: 58 [PMID: 24004921 DOI: 10.1186/2049-6958-8-58]

31 **Puente-Maestu L**, Calle M, Ortega-González A, Fuster A, González C, Márquez-Martín E, Marcos-Rodriguez PJ, Calero C, Rodríguez-Hermosa JL, Malo de Molina R, Aburto M, Sobradillo P, Alcázar B, Tirado-Conde G. Multicentric study on the beta-blocker use and relation with exacerbations in COPD. *Respir Med* 2014; **108**: 737-744 [PMID: 24635914 DOI: 10.1016/j.rmed.2014.02.009]

32 **Andrikopoulos G**, Pastromas S, Kartalis A, Toli K, Mantas I, Tzeis S, Kyrpizidis C, Olympios C, Manolis AJ, Foussas S, Kranidis A, Pras A, Pipilis A, Chryssos D, Gotsis A, Trikas A, Richter D, Alexopoulos D, Parthenakis F, Theodorakis G, Konstantinides S, Vardas P. Inadequate heart rate control is associated with worse quality of life in patients with coronary artery disease and chronic obstructive pulmonary disease. The RYTHMOS study. *Hellenic J Cardiol* 2012; **53**: 118-126 [PMID: 22484777]

33 **Boskabady MH**, Snashall PD. Bronchial responsiveness to beta-adrenergic stimulation and enhanced beta-blockade in asthma. *Respirology* 2000; **5**: 111-118 [PMID: 10894099 DOI: 10.1046/j.1440-1843.2000.00236.x]

34 **Salpeter SR**, Ormiston TM, Salpeter EE. Cardioselective beta-blockers in patients with reactive airway disease: a meta-analysis. *Ann Intern Med* 2002; **137**: 715-725 [PMID: 12416945 DOI: 10.7326/0003-4819-137-9-200211050-00035]

35 **Loth DW**, Brusselle GG, Lahousse L, Hofman A, Leufkens HG, Stricker BH. β-Adrenoceptor blockers and pulmonary function in the general population: the Rotterdam Study. *Br J Clin Pharmacol* 2014; **77**: 190-200 [PMID: 23772842 DOI: 10.1111/bcp.12181]

36 **Brooks TW**, Creekmore FM, Young DC, Asche CV, Oberg B, Samuelson WM. Rates of hospitalizations and emergency department visits in patients with asthma and chronic obstructive pulmonary disease taking beta-blockers. *Pharmacotherapy* 2007; **27**: 684-690 [PMID: 17461703 DOI: 10.1592/phco.27.5.684]

37 **Anderson WJ**, Short PM, Williamson PA, Manoharan A, Lipworth BJ. The inverse agonist propranolol confers no corticosteroid-sparing activity in mild-to-moderate persistent asthma. *Clin Sci* (Lond) 2014; **127**: 635-643 [PMID: 24938324 DOI: 10.1042/CS20140249]

38 **Short PM**, Anderson WJ, Williamson PA, Lipworth BJ. Effects of intravenous and oral β-blockade in persistent asthmatics controlled on inhaled corticosteroids. *Heart* 2014; **100**: 219-223 [PMID: 24203262 DOI: 10.1136/heartjnl-2013-304769]

39 **Rinaldi B**, Capuano A, Gritti G, Donniacuo M, Scotto Di Vettimo A, Sodano L, Rafaniello C, Rossi F, Matera MG. Effects of chronic administration of β-blockers on airway responsiveness in a murine model of heart failure. *Pulm Pharmacol Ther* 2014; **28**: 109-113 [PMID: 24769100 DOI: 10.1016/j.pupt.2014.04.005]

40 **Zhou Y**, Xu M, Zhang Y, Guo Y, Zhang Y, He B. Effects of long-term application of metoprolol and propranolol in a rat model of smoking. *Clin Exp Pharmacol Physiol* 2014; **41**: 708-715 [PMID: 24837395 DOI: 10.1111/1440-1681.12261]

41 **Guo Y**, Zhang Y, Shen N, Zhou Y, Zhang Y, Wupuer H, He B. Effects of one month treatment with propranolol and metoprolol on the relaxant and contractile function of isolated trachea from rats exposed to cigarette smoke for four months. *Inhal Toxicol* 2014; **26**: 271-277 [PMID: 24669949 DOI: 10.3109/08958378.2014.885098]

42 **Azzi M**, Charest PG, Angers S, Rousseau G, Kohout T, Bouvier M, Piñeyro G. Beta-arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. *Proc Natl Acad Sci USA* 2003; **100**: 11406-11411 [PMID: 13679574 DOI: 10.1073/pnas.1936664100]

43 **Baker JG**, Hall IP, Hill SJ. Agonist and inverse agonist actions of beta-blockers at the human beta 2-adrenoceptor provide evidence for agonist-directed signaling. *Mol Pharmacol* 2003; **64**: 1357-1369 [PMID: 14645666 DOI: 10.1124/mol.64.6.1357]

44 **Soriano-Ursúa MA**, Trujillo-Ferrara JG, Correa-Basurto J, Vilar S. Recent structural advances of β1 and β2 adrenoceptors yield keys for ligand recognition and drug design. *J Med Chem* 2013; **56**: 8207-8223 [PMID: 23862978 DOI: 10.1021/jm400471z]

45 **Amezcua-Gutierrez MA**, Cipres-Flores FJ, Trujillo-Ferrara JG, Soriano-Ursua MA. Clinical implications of recent insights into the structural biology of beta2 adrenoceptors. *Curr Drug Targets* 2012; **13**: 1336-1346 [PMID: 22812411 DOI: 10.2174/138945012802429741]

46 **Zhou Y**, Zhang Y, Guo Y, Zhang Y, Xu M, He B. β2-Adrenoceptor involved in smoking-induced airway mucus hypersecretion through β-arrestin-dependent signaling. *PLoS One* 2014; **9**: e97788 [PMID: 24905583 DOI: 10.1371/journal.pone.0097788]

47 **Peng H**, Bond RA, Knoll BJ. The effects of acute and chronic nadolol treatment on β2AR signaling in HEK293 cells. *Naunyn Schmiedebergs Arch Pharmacol* 2011; **383**: 209-216 [PMID: 21225244 DOI: 10.1007/s00210-010-0591-9]

48 **Waagstein F**, Hjalmarson A, Varnauskas E, Wallentin I. Effect of chronic beta-adrenergic receptor blockade in congestive cardiomyopathy. *Br Heart J* 1975; **37**: 1022-1036 [PMID: 1191416]

49 **Huiart L**, Ernst P, Suissa S. Cardiovascular morbidity and mortality in COPD. *Chest* 2005; **128**: 2640-2646 [PMID: 16236937 DOI: 10.1378/chest.128.4.2640]

50 **Sin DD**, Man SF. Chronic obstructive pulmonary disease: a novel risk factor for cardiovascular disease. *Can J Physiol Pharmacol* 2005; **83**: 8-13 [PMID: 15759045]

51 **Salpeter SR**, Ormiston TM, Salpeter EE. Cardiovascular effects of beta-agonists in patients with asthma and COPD: a meta-analysis. *Chest* 2004; **125**: 2309-2321 [PMID: 15189956]

52 **Du Q**, Sun Y, Ding N, Lu L, Chen Y. Beta-blockers reduced the risk of mortality and exacerbation in patients with COPD: a meta-analysis of observational studies. *PLoS One* 2014; **9**: e113048 [PMID: 25427000 DOI: 10.1371/journal.pone.0113048]

53 **Short PM**, Lipworth SI, Elder DH, Schembri S, Lipworth BJ. Effect of beta blockers in treatment of chronic obstructive pulmonary disease: a retrospective cohort study. *BMJ* 2011; **342**: d2549 [PMID: 21558357 DOI: 10.1136/bmj.d2549]

54 **Lee DS**, Markwardt S, McAvay GJ, Gross CP, Goeres LM, Han L, Peduzzi P, Lin H, Dodson JA, Tinetti ME. Effect of β-blockers on cardiac and pulmonary events and death in older adults with cardiovascular disease and chronic obstructive pulmonary disease. *Med Care* 2014; **52** Suppl 3: S45-S51 [PMID: 24561758 DOI: 10.1097/MLR.0000000000000035]

55 **Tavazzi L**, Swedberg K, Komajda M, Böhm M, Borer JS, Lainscak M, Robertson M, Ford I. Clinical profiles and outcomes in patients with chronic heart failure and chronic obstructive pulmonary disease: an efficacy and safety analysis of SHIFT study. *Int J Cardiol* 2013; **170**: 182-188 [PMID: 24225201 DOI: 10.1016/j.ijcard.2013.10.068]

56 **Chen J**, Radford MJ, Wang Y, Marciniak TA, Krumholz HM. Effectiveness of beta-blocker therapy after acute myocardial infarction in elderly patients with chronic obstructive pulmonary disease or asthma. *J Am Coll Cardiol* 2001; **37**: 1950-1956 [PMID: 11401137 DOI: 10.1016/S0735-1097(01)01225-6]

57 **Gottlieb SS**, McCarter RJ, Vogel RA. Effect of beta-blockade on mortality among high-risk and low-risk patients after myocardial infarction. *N Engl J Med* 1998; **339**: 489-497 [PMID: 9709041 DOI: 10.1056/NEJM199808203390801]

58 **van Gestel YR**, Hoeks SE, Sin DD, Welten GM, Schouten O, Witteveen HJ, Simsek C, Stam H, Mertens FW, Bax JJ, van Domburg RT, Poldermans D. Impact of cardioselective beta-blockers on mortality in patients with chronic obstructive pulmonary disease and atherosclerosis. *Am J Respir Crit Care Med* 2008; **178**: 695-700 [PMID: 18565952 DOI: 10.1164/rccm.200803-384OC]

59 **Rutten FH**, Zuithoff NP, Hak E, Grobbee DE, Hoes AW. Beta-blockers may reduce mortality and risk of exacerbations in patients with chronic obstructive pulmonary disease. *Arch Intern Med* 2010; **170**: 880-887 [PMID: 20498416 DOI: 10.1001/archinternmed.2010.112]

60 **Heart Failure Society of America**. Executive summary: HFSA 2006 Comprehensive Heart Failure Practice Guideline. *J Card Fail* 2006; **12**: 10-38 [PMID: 16500578 DOI: 10.1016/j.cardfail.2005.12.001]

61 **Morales DR**, Guthrie B, Lipworth BJ, Donnan PT, Jackson C. Prescribing of β-adrenoceptor antagonists in asthma: an observational study. *Thorax* 2011; **66**: 502-507 [PMID: 21459857 DOI: 10.1136/thoraxjnl-2011-200067]

62 **Callaerts-Vegh Z**, Evans KL, Dudekula N, Cuba D, Knoll BJ, Callaerts PF, Giles H, Shardonofsky FR, Bond RA. Effects of acute and chronic administration of beta-adrenoceptor ligands on airway function in a murine model of asthma. *Proc Natl Acad Sci USA* 2004; **101**: 4948-4953 [PMID: 15069206 DOI: 10.1073/pnas.040045210]

63 **Lin R**, Peng H, Nguyen LP, Dudekula NB, Shardonofsky F, Knoll BJ, Parra S, Bond RA. Changes in beta 2-adrenoceptor and other signaling proteins produced by chronic administration of 'beta-blockers' in a murine asthma model. *Pulm Pharmacol Ther* 2008; **21**: 115-124 [PMID: 17689122 DOI: 10.1016/j.pupt.2007.06.003]

64 **Hanania NA**, Singh S, El-Wali R, Flashner M, Franklin AE, Garner WJ, Dickey BF, Parra S, Ruoss S, Shardonofsky F, O'Connor BJ, Page C, Bond RA. The safety and effects of the beta-blocker, nadolol, in mild asthma: an open-label pilot study. *Pulm Pharmacol Ther* 2008; **21**: 134-141 [PMID: 17703976 DOI: 10.1016/j.pupt.2007.07.002]

65 **Short PM**, Williamson PA, Anderson WJ, Lipworth BJ. Randomized placebo-controlled trial to evaluate chronic dosing effects of propranolol in asthma. *Am J Respir Crit Care Med* 2013; **187**: 1308-1314 [PMID: 23593932 DOI: 10.1164/rccm.201212-2206OC]

**P-Reviewer:** Pessi T, Soriano-Ursua M **S-Editor:** Ji FF **L-Editor: E-Editor:**

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**Figure 1 Long-term usage of β-agonists will result in a loss of Broncho-Protective Effect where β-adrenoceptors desensitization occurs.** Broncho-Protective Effect is conferred when β-agonist binds to β2-AR, activating adenyl cyclase through Gαs, leading to an increase in cAMP levels. The surge in cAMP in turn activates PKA which phosphorylates myosin light chain to inhibit contraction. PKA also activates K+ channels, inducing membrane hyperpolarization which counteracts electrical excitation leading to contraction. Chronic use of β-agonist will lead to a loss of this Broncho-Protective Effect due to the uncoupling of Gαs from β2-AR, phosphorylation by PKA/GRK and the binding of β-arrestin which leads to internalization, downregulation and desensitization towards β-agonist[15,27,28]. PKA: Protein kinase A; GRK: G-protein receptor kinases; AR: Adrenoceptors.



**Figure 2 Potential therapeutic benefits by chronic β-blocker usage in obstructive airway diseases observed in animal and clinical studies[12,40,46,47,64,65].** AR: Adrenoceptors; TNF: Tumor necrosis factor; IL: Interlukin.



**Figure 3 Acute and chronic inverse agonist treatment in obstructive airway diseases.** It was shown in cell and animal models that acute treatment of β-blockers induced a partial agonist response that led to an increase in MUC5AC production *via* β-arrestin2 which serves as a multi-protein scaffold, activating ERK1/2 and p38 mitogen-activated protein kinase (MAPK), resulting in mucus hypersecretion and increased airway resistance response to methacholine. However, chronic treatment of β-blockers led to a reduction in mucus secretion, decreased airway hyperresponsiveness and reduced inflammation, through the non-canonical β-arrestin2-mediated signaling induced by inverse agonism of β2-adrenoceptors[46]. The differential response could be due to the binding of ligand to a shallower secondary binding site exposed only when an adequate conformational state is obtained as proposed by Soriano-Ursúa *et al*[44], however more work need to be done to validate the mechanism.