**CONTROVERSIAS EN EL TRATAMIENTO FARMACOLÓGICO DE LA EPOC**

**Association of β₂-adrenergic agonists and tiotropium: is the combination justified?**

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**Introduction**

Current treatment for chronic obstructive pulmonary disease (COPD) is aimed at increasing airflow, decreasing respiratory symptoms (particularly dyspnea), decreasing exacerbations, and improving the quality of life. Consequently, it is not surprising that both the Global Initiative for Obstructive Lung Disease (GOLD) guidelines¹, and the recent American Thoracic Society (ATS)/European Respiratory Society (ERS) position paper recommend for moderate-to-very severe COPD the use of long-acting bronchodilators (e.g., salbutamol, terbutaline, ipratropium or oxitropium) or long-acting (e.g., formoterol, salmeterol, or tiotropium). Both the GOLD Update and the ATS/ERS position paper recommend, for moderate-to-severe COPD, the use of regular treatment with long-acting bronchodilators rather than short acting bronchodilators, with the choice depending on the availability of medication and the patient’s response²,³.

For patients whose conditions are not sufficiently controlled by monotherapy, these guidelines highlight that combining medications of different classes, in particular an inhaled anticholinergic with a β₂-AR agonist, seems a convenient way of delivering treatment and obtaining better results²,⁴. This includes better lung function and improved symptoms.

**Rationale for combining β₂-adrenergic agonists and anticholinergic agents**

Postganglionic, parasympathetic-cholinergic nerves innervate the airways. When activated, these nerves are capable of obliterating the lumen of small bronchi and bronchioles, and markedly increasing airway resistance in larger, cartilaginous airways, by secretion of the bronchoconstricting mediator acetylcholine (ACh), which causes activation of muscarinic receptors at the level of the target cells, such as bronchial smooth muscle and goblet cells⁴,⁵. Conversely, sympathetic nerves may control tracheobronchial blood vessels, but no innervation of human airway smooth muscle has been demonstrated. β₂-ARs, however, are abundantly expressed on human airway smooth muscle and activation of these receptors causes its relaxation⁶.

Bronchodilation may, therefore, be obtained either by stimulating the β₂-ARs with β₂-AR agonists, or by inhibiting the action of ACh at muscarinic receptors with anticholinergic agents. In any case, anticholinergics are more likely to decrease central airway resistance, although there are muscarinic receptors that are expressed in the smooth muscle of small airways which do not appear to be innervated by cholinergic nerves, and β₂-AR agonists have a greater effect on peripheral airway resistance in patients with COPD⁷. It is reasonable to postulate that attempts to reduce bronchoconstriction through two distinct mechanisms (anticholinergic and sympathomimetic) with a different prevalent site of action may maximize bronchodilator response.

Interestingly, the presence of small dense-cored vesicles containing adrenergic nerve varicosities, occasionally in close proximity to morphologically characteristic cholinergic nerve-endings, has been identified in human airways⁸. Early research papers based on force measurement alone suggested that stimulation of β₂-ARs inhibits cholinergic neurotransmission⁹, most probably by the release of inhibitory prostaglandins from the airway mucosa. However, interpretation of these data is seriously hampered by the large postjunctional effects of β₂-AR agonists. Zhang et al.¹⁰ were the first to report an excitatory β₂-AR in airway parasympathetic nerves. After their report in studies on the horse, this receptor was subsequently reported in guinea pig¹¹ and in human airway parasympathetic nerves¹². Activation of β₂-ARs by isoproterenol, by the racemic mixture of specific β₂-AR agonists such as salbutamol and formoterol or by R-
enitomers of β2-agonists can increase ACh release in a concentration-dependent manner\(^1\). Consistent with the results of β2-AR stimulation\(^12\), direct activation of the β2-AR-coupled Gs protein by cholera toxin, which increases the activity of adenylyl cyclase, caused an increase in ACh release in epithelium-denuded guinea pig trachealis\(^13\). In airway smooth muscle cells, however, stimulation of Gs protein directly opens large Ca\(^{2+}\)-activated potassium (K\(_{Ca}\)) channels\(^13\), which has been found to decrease ACh release in guinea pig trachealis\(^13\). Recently, Brichetto et al\(^14\) have confirmed that β2-AR agonists attenuate cholinergic neurotransmission in the isolated bovine trachealis model and this happens by a mechanism not involving cAMP but K\(_{Ca}\) channels.

Whatever the type of interaction between the two systems may be, combining β2-AR agonists and anticholinergic agents is pharmacologically useful. In fact, in the first case, the addition of a β2-AR agonist decreases the release of ACh because of the modulation of cholinergic neurotransmission by prejunctional β2-ARs and, consequently, amplifies the bronchial smooth muscle relaxation directly induced by the anticholinergic agent. On the contrary, in the second circumstance, the addition of an anticholinergic agent can reduce the peripheral bronchoconstrictor effects of ACh, whose release has been facilitated by the β2-AR agonist, and in this manner can amplify the bronchodilatation elicited by the β2-AR agonist through the direct stimulation of smooth muscle β2-ARs.

**Combination therapy with ipratropium and a β2-agonist**

It has been documented that standard doses of short acting β2-agonists do not give optimal results in patients with COPD and that an anticholinergic agent gives additional bronchodilatation\(^8\). In addition, the reproducibility of responsiveness to bronchodilators in patients with COPD is improved when the pulmonary function test is performed using a combination of ipratropium and salbutamol\(^15\). In effect, although a few articles question the value of combination therapy with ipratropium bromide and a β2-AR agonist\(^16,22\), several large trials suggest that the two drugs, both at low doses\(^17\) and at high doses\(^18,23\), have complementary, or additive, bronchodilator actions without any increase in the incidence of adverse reactions, which makes them an excellent combination for the treatment of COPD. The inclusion of a second agent of a different class in a pharmacologic treatment regimen is associated with a lower rate of exacerbations in COPD\(^24\). The overall result is lower total treatment costs and improved cost-effectiveness.

The introduction of long acting β2-AR agonist bronchodilators gives physicians additional therapeutic options for COPD. Both salmeterol and formoterol appear to be more effective than short acting β2-AR agonists\(^25\) and in patients with stable COPD they are more effective than anticholinergic agents\(^26,27\).

Two published studies that had evaluated small cohorts suggested that there is no substantial additive effect when a long-acting β2-AR agonist is combined with ipratropium bromide given acutely at the clinically recommended dose (40 µg) in patients with COPD\(^28,29\), but it must be stressed that the dose of ipratropium bromide needed to produce near maximal bronchodilatation is several times higher than the customary dosage\(^30\). In fact, the results of some studies suggest that higher than normal doses of an anticholinergic drug must be used for further relief of bronchospasm in patients with COPD when a single conventional inhaled dose of formoterol\(^31\) or salmeterol\(^32\) is given first.

In any case, van Noord et al\(^33\) demonstrated that a 12-week treatment with salmeterol 50 µg twice daily plus ipratropium bromide 40 µg four times daily was more effective than salmeterol 50 µg twice daily in improving forced expiratory volume in 1 second (FEV\(_1\)) and specific airway conductance. This study was the first to point out that the association of a long acting β2-AR agonist and an anticholinergic agent is useful in the long term therapy of stable COPD.

Subsequently, D’Ursio et al\(^34\) not only confirmed this therapeutic possibility, but even documented that the addition of formoterol (12 µg twice daily) to ipratropium bromide (40 µg four times a day) is more effective than the addition of salbutamol (200 µg four times a day) in patients with COPD who required combined bronchodilator therapy. This finding clearly indicates that long-acting β2-AR agonists may represent the most effective option for combination therapy with an antimuscarinic agent.

**The functional impact of combining long-acting bronchodilators**

Clinical studies show that tiotropium administered 18 µg once daily improves lung function over its 24 h dosing interval, as shown by FEV\(_1\), forced vital capacity

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Fig. 1. Schematic presentation of the potential alternative role of β2-adrenergic (AR) in the pre-synaptic control of acetylcholine (ACh) release from airway parasympathetic nerve endings. A: circulating adrenaline; β1-AR: β1-adrenoceptor; M1 and M3: muscarinic, M1 or M3 receptors, (→) neuronal activity; ↔ stimulatory effect; ♂ inhibitory effect.
formoterol 12 µg (FOR), tiotropium 18 µg (TIO), and tiotropium 18 µg + formoterol 12 µg (TIO + FOR). p < 0.01; **p < 0.001 versus baseline. (Data from Cazzola et al.)*

(FVC), peak expiratory flow rate (PEFR) and measures of hyperinflation and provides superior spirometric improvements compared with ipratropium 40 µg four times daily.** Considering this important finding, in an elegant review in which the pharmacological actions of the long-acting β2-AR agonists and a long-acting muscarinic antagonist (tiotropium bromide) were summarized, Tennant et al.** highlighted the need for investigating the combination of tiotropium bromide with a long-acting β2-AR agonist. Afterwards, Tashkin and Cooper** used the traditional method of integrating research studies that, unfortunately, does not allow to determine if the differences between the study outcomes are due to chance, to inadequate study methods or to systematic differences in the characteristics of the studies, emphasized the advantage of tiotropium on long-acting β2-agonists, and suggested adding salmeterol or formoterol to tiotropium bromide, at least in patients suffering from COPD with more severe symptoms (stage III or IV of the GOLD classification). However, Cazzola and Matese** in an accompanying editorial comment highlighted that no published study has documented the superiority of tiotropium over formoterol, although two studies, specifically designed to explore the potential differences between tiotropium and salmeterol, seem to indicate a greater efficacy of tiotropium than long-acting β2-AR agonists.** The different pharmacodynamic profile of formoterol when compared to salmeterol** might induce a different type of bronchodilatory effect, mainly if one considers onset of action or peak bronchodilatation. This may lead to a different conclusion when comparing formoterol with salmeterol. Moreover, it is not known if the combination of a long-acting β2-AR agonist and a long-acting antimuscarinic agent provides further advantages in terms of bronchodilatation over either drug alone, nor if the choice of the specific long-acting β2 agonist to be used is trivial.

**Acute functional effect of combining formoterol or salmeterol and tiotropium**

Some studies have tried to give an answer to these questions. A pilot investigational trial, which enrolled 20 outpatients clinically diagnosed with stable COPD and a mean baseline FEV1 of 0.87 l (95% confidence interval [CI], 0.70-1.04) and FVC of 1.49 l (95% CI, 1.30-1.69), showed that 12 µg formoterol, either alone or in combination with 18 µg tiotropium, elicited a significantly faster onset of action (the change in FEV1, 10 min after inhalation of formoterol alone [0.088 l; 95% CI, 0.049-0.127] was greater than that induced by tiotropium alone [0.039 l; 95% CI, 0.006-0.071], but not than that elicited by formoterol + tiotropium [0.085 l; 95% CI, 0.044-0.126]) (fig. 2 A). Moreover, this study also documented a trend for a greater maximum bronchodilatation with combination than with formoterol or tiotropium alone (the mean maximum increases in FEV1 from pre-dosing value on each of the dosing days were 0.192 l [95% CI, 0.125-0.259] for formoterol, 0.176 [95% CI, 0.100-0.253] for tiotropium, and 0.210 l [95% CI, 0.158-0.261] for the combination and occurred two hours after formoterol and three hours after inhalation of tiotropium and the combination, but the difference between treatments was not significant (p = 0.475)) (fig. 2 B). At twenty four hours, mean FEV1 continued to be significantly higher than pre-dosing value following tiotropium 0.084 l, 95% CI, 0.003-0.134; p = 0.003 and formoterol + tiotropium 0.088 l; 95% CI, 0.002-0.173; p = 0.045), but did not achieve significance for formoterol alone (0.058 l; 95% CI, 0.000-0.117; p = 0.051) (fig. 2 B). However, at this time point, the differences between treatments were not significant (p = 0.731). The failure to show a statistically significant difference between treatments when we explored the maximum bronchodilatation and the duration of action was likely associated with an insufficient statistical power in the study. We believe that there was a possibility of a type II error, which supported the lack of significance for formoterol alone. A larger study would achieve statistical significance.

The results of this study indicate that formoterol and tiotropium have different profiles (formoterol has a faster onset of action and greater bronchodilatating effect, tiotropium has a longer duration of action, which allows...
for once daily administration) that make both agents attractive alternatives in the treatment of stable COPD. Moreover, the two drugs appear complementary: tiotropium ensures prolonged bronchodilation, whereas formoterol provides fast onset and a greater peak effect. However, because formoterol is given twice daily, but tiotropium is required only once daily, and results of our study do not allow suggesting the once daily dosing of formoterol, the challenge is to develop a combined inhaler that can be employed on a daily basis. The pharmacodynamic characteristics of salmeterol might permit the once daily contemporaneous administration of the two drugs that could simplify the therapy.

In a further study that has enrolled 20 outpatients with stable COPD and a mean baseline FEV\textsubscript{1} of the two drugs, the potential of salmeterol to increase FEV\textsubscript{1} with stable COPD, but exclude the once-daily co-administration of both salmeterol and tiotropium in patients suffering from COPD, there is a fundamental question of whether high local concentrations after inhalation of the two drugs that could simplify the therapy.

**Fig. 3. Mean changes (± SE) in FEV\textsubscript{1} from pre-dosing value on each of the dosing days up to 90 min (A) and up to 24 h (B) after inhalation of salmeterol 50 µg (SALM), tiotropium 18 µg (TIO), and salmeterol 50 µg + tiotropium 18 µg (SALM + TIO).**

**A.** Minutes

**B.** Hours

These findings support the possibility of combining tiotropium and salmeterol in patients suffering from stable COPD, but exclude the once-daily co-administration of the two drugs. The potential of salmeterol to increase its onset of action when combined with tiotropium is worthy of attention considering that both agents elicit a slow onset of action. Ethier et al\textsuperscript{48} speculated that salmeterol has a low affinity binding interaction with the muscarinic receptorG-protein complex itself or reversibly alters the plasma membrane environment surrounding the complex. In any case, these effects of salmeterol did not depend on stimulation of \( \beta\)-ARs. If this finding were also true for human airways, we could speculate that salmeterol reduces the bronchoplastic activity of endogenous AChs, without influencing the effect of tiotropium. Salmeterol has also high affinity for muscarinic receptors\textsuperscript{47} and this indicates a potential for amplifying the action of tiotropium. However, the question of whether high local concentrations after inhalation of salmeterol could contribute to its therapeutic effects by antagonism with AChs on muscarinic receptors remains to be answered.

**Functional effect of a regular treatment with formoterol or salmeterol and tiotropium**

Although these results indicate that a combination of tiotropium and a long-acting \( \beta\)-AR agonist is more effective than single drugs alone in inducing bronchodilatation in patients suffering from COPD, there is a fundamental question of whether high local concentrations after inhalation of salmeterol could contribute to its therapeutic effects by antagonism with AChs on muscarinic receptors remains to be answered.

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mental aspect of these studies, the evaluation of the impact of the three treatments only after acute administration, that must be taken into account. Acute administration is, in fact, a potential bias because it is well known that FEV₁ steady state with tiotropium is reached within forty eight hours, while continued improvements in FVC can be expected over or beyond the first week of therapy. The progressive increases in FVC beyond forty eight hours suggest that maintenance bronchodilator therapy is required to achieve maximal changes in hyperinflation.

Data of a trial that compared the lung function response of the free combination of 18 µg tiotropium plus 12 µg formoterol once daily with 18 µg tiotropium once daily and 12 µg formoterol twice daily, with all treatments administered for 6-week periods, in 66 patients with moderate-to-severe stable COPD, documented that the free combination of once daily tiotropium plus formoterol was better than either of the single drugs for most of the spirometric endpoints. Tiotropium once daily was superior to formoterol twice daily during the daytime. However, during the night-time, tiotropium and formoterol provided similar bronchodilation. In particular, the average FEV₁ area under the curve (AUC₁₋₂₄) was 85 ml after tiotropium, 62 ml after formoterol and 160 ml after tiotropium + formoterol, the average FEV₁/AUC₁₋₂₄ was 127 ml after tiotropium, 86 ml after formoterol and 234 ml after tiotropium + formoterol, whereas the average FEV₁/AUC₁₋₂₄ was 43 ml after tiotropium, 38 ml after formoterol and 86 ml after tiotropium + formoterol (fig. 4). All FEV₁/AUC after tiotropium + formoterol were significantly (p < 0.05) larger than that of tiotropium or formoterol alone. The daytime, but not the night-time, use of rescue salbutamol was significantly reduced in patients receiving the combination of tiotropium + formoterol (p < 0.05 versus either drug alone). The documentation that tiotropium was more active than formoterol in daytime but not in night-time was, in our opinion, in agreement with the fact that the activity in the sympathetic system appears to be prominent during the day as reflected by the peak located around noon of the urinary catecholamine excretion, whereas the vagal system appears to be prominent during the remainder of the day. In any case, these results indicate that once daily combination therapy of tiotropium + formoterol is safe and provides significant additive effects in patients with moderate-to-severe COPD. Moreover, they suggest that once daily administration of the two drugs could be a possibility in the treatment of stable COPD. A bronchodilator-mediated symptom benefit of the once daily combination is also reflected in significant decrease in salbutamol use as rescue therapy.

However, another trial that explored tiotropium maintenance therapy in 91 patients with COPD and the forty four hour spirometric benefit of adding once or twice daily formoterol during two-week treatment periods, documented that add-on therapy of a second dose. The average FEV₁/AUC₁₋₂₄ was 80 ml after tiotropium, 162 ml after tiotropium + formoterol once daily and 198 ml after tiotropium + formoterol twice daily, the average FEV₁/AUC₁₋₂₄ was 125 ml after tiotropium, 238 ml after tiotropium + formoterol once daily and 241 ml after tiotropium + formoterol twice daily, whereas the average FEV₁/AUC₁₋₂₄ was 37 ml after tiotropium, 89 ml after tiotropium + formoterol once daily and 156 ml after tiotropium + formoterol twice daily (fig. 5). FEV₁/AUC₁₋₂₄ of tiotropium + formoterol twice
The best strategy for adding long-acting β₂-agonists and long-acting anticholinergics

These findings raise an important question. What is the best strategy for adding long-acting β₂-AR agonists and long-acting anticholinergics in COPD? Some studies have examined various strategies for adding short-acting β₂-AR agonists and anticholinergics in COPD. Unfortunately, they have provided conflicting results. Rennard\(^\text{52}\) has correctly stressed that this may depend on the nature of the circumstances of the patient at the time when the study was carried out and may depend on the design by which the studies were conducted and the drugs administered.

In one of our previous trials, which was the first to our knowledge that compared a long-acting β₂-AR agonist and an anticholinergic agent given by sequential inhalation at the recommended dosages, we documented that the sequential administration of formoterol and oxis tropium bromide induced an improvement in pulmonary function in a population of COPD patients similar to that examined in the present trial.\(^\text{54}\) However, prior administration of the long-acting β₂-AR agonist allowed a response to the anticholinergic drug, which was higher than that observed when inhalation of oxis tropium was preceded by that of formoterol.

In order to explore whether this finding is true even when tiotropium is used instead of oxis tropium, we have examined the potential of an additive effect of a recommended dose of second long-acting bronchodilator (tiotropium or formoterol) in COPD patients under regular treatment with a long-acting bronchodilator of a different class (formoterol or tiotropium, respectively). We conducted a randomized, crossover trial in 20 patients with 18 μg tiotropium once daily and 12 μg formoterol twice daily over a five-day period for each drug, with a ten-day washout period.\(^\text{55}\) At the end of each period, patients inhaled both drugs separated by 180 min in alternate sequence. Thirty minutes after inhalation of the last dose of tiotropium, there was a statistically significant increase of 0.099 l (95% CI, 0.062-0.138) in FEV\(_1\), over baseline (p < 0.0001) (fig. 6). The same was observed after inhalation of the last dose of formoterol (0.166 l; 95% CI, 0.064-0.268, p < 0.0002). The mean maximal change in FEV\(_1\) over pre-inhalation of the second drug value was 0.081 l (95% CI, 0.029-0.133) after tiotropium → formoterol and 0.054 l (95% CI, 0.016-0.092) after formoterol → tiotropium. The mean maximal change in FEV\(_1\) over baseline was 0.519 l (95% CI, 0.361-0.676) in group A and 0.495 l (95% CI, 0.307-0.683) in group B. The mean maximal change in FVC over pre-inhalation of the second drug value was 0.159 l (95% CI, 0.048-0.270) after tiotropium-formoterol and 0.175 l (95% CI, 0.083-0.266) after formoterol → tiotropium.

These results suggest that supplementing a second different long-acting bronchodilator to a regularly administered long-acting bronchodilator seems to be to the patient’s advantage in terms of bronchodilation. We cannot exclude that the greater bronchodilatory response that we observed when a second bronchodilator was given after the first one may be justified by a carry over effect, considering that both formoterol and tiotropium are long-lasting bronchodilators. Nonetheless, it is well known that the mean peak bronchodilation with both formoterol and tiotropium in COPD patients is reached after two to three hours\(^\text{56}\) and we have documented that the addition of a second bronchodilator three hours after the inhalation of the first agent, could amplify the maximum bronchodilation of the first agent. This result seems to be important because it indicates the possibility...
that a patient who is unable to perceive bronchodilation or must perform an exercise could use a second long-acting bronchodilator that will assure a long-lasting effect. In any case, it must be highlighted that significant improvement in pulmonary function has been achieved by adding tiotropium or formoterol at the recommended dosages in patients already in regular treatment with formoterol or tiotropium, respectively, with no statistically significant difference between the different sequences. This finding supports Rennard’s opinion that treatment can be initiated with an agent from any of the available classes. If symptomatic control is inadequate, an agent from another class can be added.

Conclusions

At present time, there is no clear documentation that tiotropium is superior to formoterol or the contrary. At the recommend doses for COPD therapy, formoterol twice daily and tiotropium once daily induce comparable night time bronchodilation after regular treatment. A combination of tiotropium and a long-acting β₂-AR agonist is more effective than single drugs alone in inducing bronchodilation and bronchodilator-mediated symptoms in patients suffering from COPD. Add-on therapy of formoterol in the morning to maintenance therapy with tiotropium significantly improves FEV₁ and IC for more than twelve hours in patients with moderate-to-severe COPD. Add-on therapy of a second formoterol dose administered in the evening produces a further increase in average FEV₁ and IC, but not to trough IC. All these findings support, in our opinion, the possibility of combining tiotropium and formoterol, and likely salmeterol, in patients suffering from stable COPD, but exclude the once-daily administration of the two drugs in combination within a single inhaler. It must be highlighted that a combination of tiotropium and formoterol is more effective when administered in the evening compared with morning dosing, in view of the circadian variation of bronchial tone.

Because long-acting β₂-AR agonists are given twice daily but tiotropium bromide is required only once daily, the challenge is now to develop a combined inhaler that can be employed on a once daily basis. The incorporation of once daily dosing is an important strategy to improve compliance since it is a regime preferred by most patients.

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