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Albuterol enantiomers: pre-clinical and clinical value?

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1. ABSTRACT

Albuterol has been used in the acute treatment of asthma exacerbations for over 25 years. Its cost is low, and delivery can be tailored to allow dose-effect titration. Like other beta-2-adrenergic receptor agonists, it can exist as a racemate of two enantiomers, one active [(R)-albuterol], and one traditionally considered inert [(S)-albuterol]. Basic investigations in airway cells and models from animals and humans have shown that (R)-albuterol, in both racemic and single enantiomer formulations, produces changes consistent with both relaxation of airway smooth muscle cells, and the reduction of inflammation. In contrast, (S)-albuterol typically has produces effects opposite to those of (R)-albuterol, i.e., antagonistic to the beneficial desired effects. Coupled with the fact that (S)-albuterol can persist 12 times longer than (R)-albuterol within the human circulation, findings suggest that paradoxical effects, sometimes seen with chronic racemic albuterol use, are due to (S)-albuterol. A number of clinical studies, to date, have been generally consistent with these findings; however, overwhelming evidence for clinical superiority of the (R)-albuterol single enantiomer over that within racemic albuterol remains to be obtained.

2. INTRODUCTION

Albuterol is a synthetic short-acting beta-2-adrenergic receptor agonist, used in the treatment of asthma, for over twenty-five years. With the advent of metered-dose inhalers, it is easily self-administered, its cost is relatively low, and it has an acceptable shelf-life requiring almost no special handling or maintenance. Importantly, its use can be patient-titrated to effect, typically bringing rapid relief of bronchoconstriction within minutes of its administration.

With few exceptions, albuterol and other beta-2-adrenergic receptor agonists currently utilized for the treatment of asthma are racemic mixtures: 50:50 mixtures of the active and inactive enantiomers of the drug. This 50:50 mixture is a consequence of organic chemical synthesis, in which a symmetrical intermediate is present, and results in compounds having the same chemical formula, but with exactly opposite chemical structures. However, biologic systems are generally quite selective as to which enantiomer is utilized for a given function. For example, humans employ L-amino acids and D-sugars, and not their opposing counterparts, for most biologic processes. The beta-2-adrenergic receptors are likewise stereospecific with respect

to their ligands: for albuterol, the (R)-enantiomer binds with 100 times greater affinity than (S)-albuterol, and consequently potentiates cAMP generation more profoundly (1). As a result of this significant difference in affinity, the (S)-albuterol has been generally considered to be inert with regard to beta-2-adrenergic receptor-mediated mechanisms. However, as indicated below, accumulating basic evidence suggests that (S)-albuterol is not inactive, and may produce paradoxical intracellular effects that counter beneficial mechanistic and therapeutic effects of (R)-albuterol within racemic albuterol.

3. THE BETA-AGONIST CONTROVERSY IN ASTHMA

Asthma severity is associated with the frequency of exacerbations, and the disruption of quality of life attributable to the disease (2). The beta-agonist controversy, however, is more consequential than simple under-treatment of a complex disease (3). Despite improved treatment regimens, some patients with asthma experience "paradoxical worsening" of their asthma with the continued use of albuterol and other beta-2-adrenergic receptor agonists, and in some instances, death (4-6). Furthermore, intensive use of beta-2-adrenergic receptor agonist bronchodilators is significantly more likely in cases of death from asthma. Indeed, blood albuterol levels are 2.5 times higher in those instances, suggesting that (over)use of beta-2-adrenergic receptor agonists is associated with death from asthma, even though the beta-2-adrenergic receptor agonists may or may not be causality related (6). One important characteristic of the beta-agonist paradox is bronchospasm following beta-agonist administration, most being reported since 1973 (7-13). A 1991 review of some of those earlier studies suggested that up to 4-5% of asthmatics suffered from paradoxical bronchospasm (14); however, with increased use of both short- and long-acting beta-2-adrenergic receptor agonists in recent years, this number may actually be greater.

The exact cause of these paradoxical phenomena remains unclear. One theory is that inhaled beta-2-adrenergic receptor agonists may produce desensitization of the beta-2-adrenergic receptor (15), which may significantly reduce bronchodilation with repeated administration of albuterol. Another theory is that the overuse of beta-2-adrenegic receptor agonists is associated with asthma mortality through an indirect effect, namely that prevention of bronchoconstriction fails to treat the underlying pathogenesis associated with airway inflammation, masking true disease severity, resulting in under-treatment of the disease (16). However, there is also some indirect pre-clinical evidence (17), and some relevant clinical evidence, that racemic mixtures of albuterol containing the (S)-albuterol enantiomer could contribute to paradoxical effects observed clinically.

One possible basis for adverse effects of albuterol and other beta-2-adrenergic receptor agonists is based within its stereochemistry. Earlier reviews have presented that beta-2-adrenergic receptor agonists exist in two stereoisomeric, or enantiomeric forms, having different molecular structures, and confering differing biological properties and pharmacologic effects (18, 19). Based on those differences, those reviews recommended additional scientific investigation to elucidate and distinguish the differential effects those enantiomers (18, 19). Subsequently, studies of these compounds have repeatedly shown significant differences in many systems studied. Parenthetically, the fact that the Federal Drug Administration of the United States no longer allows routine approval of drugs for use containing both the eutomer (the form with biologic activity) and distomer (the supposed non-active, or inert, enantiomer) (20), would suggest that the potential for adverse effects of (S)-albuterol within racemic mixtures of albuterol be further considered and investigated.

4. EFFECT OF BETA-2-ADRENERGIC RECEPTOR AGONISTS IN ANIMAL AND HUMAN MODEL SYSTEMS

4.1. Overview of preclinical data

Preclinical data by topic area is reviewed in depth in other reviews associated with this review; therefore, we highlight data from a variety of studies here, and attempt to give an aggregate overview of pre-clinical results. It is apparent that in whole animals *in vivo*, and *in vitro* systems, that (R)-albuterol, whether within racemic albuterol, or administered alone, produces tissue and cellular effects mediated by ligation of the beta-2-adrenergic receptor. For example, a review of studies in guinea pigs has been provided previously by Page and Morley (21), emphasizing results in an animal model with a histaminic allergy base, similar to humans. A consistent theme from those studies, and several outlined below, was the positive effect of (R)-albuterol on bronchodilation and suppression of inflammation. Furthermore, much of the pre-clinical data also suggest that (S)-albuterol can have effects counter to (R)-albuterol. Many of the early studies were focused on the effects of enantiomers on ASM contractile responses; however, more recent data has focused upon airway inflammation. Collectively, the data suggest that: 1) (R)-albuterol effects occur due to beta-2-adrenergic receptor mechanisms, which would be expected, 2) the effects of (S)-albuterol are often the opposite of those of (R)-albuterol, and 3) the effects of (R)-albuterol can be blunted in the presence of (S)-albuterol.

4.2. Key preclinical enantiomer data on airway contractile function

4.2.1. *In vitro* animal models of airway contractility

In carbachol-stimulated bovine ASM cells, (R)-albuterol (100 micromolar) has been reported to inhibit shortening and decrease intracellular calcium concentrations (22), suggesting an ASM relaxing effect through a well-established beta-2-adrenergic receptor mechanism. Conversely, 100 micromolar (S)-albuterol increased intracellular calcium and produced significant shortening of those cells, suggesting opposing effects of the distomer on contractility. These results are consistent with the opposing effects of albuterol enantiomers mentioned above, but due to the high concentrations involved, can be viewed as being only pharmacologically relevant, rather than physiologically relevant. Importantly, a subsequent study showed that lower, and physiologically-relevant, concentrations of (R)-albuterol

ranging from 5nM to 1 micromolar, also decreased intracellular calcium, while both (S)-albuterol and racemic albuterol increased intracellular Ca^{2+} levels (23). Those data confirmed the earlier data of Penn (1), again consistent with the fact that (R)-albuterol had 100-times greater affinity for the beta-2-adrenergic receptor, as compared with (S)-albuterol (23). Furthermore, (S)-albuterol increased intracellular levels of inositol-1,4,5-triphosphate (IP_3), a key intermediate in the muscarinic M_3 receptor signaling pathway that produces ASM contraction. Interestingly, atropine inhibited the (S)-albuterol–mediated increases in intracellular Ca^{2+}, but were unaffected by the potent beta-2-adrenergic receptor antagonist ICI-118,551. Collectively, those findings have been taken by some to suggest potential independence of (S)-albuterol effects from binding to the beta-2-adrenergic receptor, with the muscarinic M_3 receptor being suggested as an alternative receptor promoting the observed effects. Findings reported in equine tracheal tissue strips *in vitro* were consistent with those in the bovine model, such that (R)-albuterol inhibited contractions, and (S)-albuterol was found to have pre-junctional effects resulting in increased acetylcholine release from parasympathetic neurons. Again, those effects represented yet another potential cholinergic mechanism through which (S)-albuterol may oppose effects of (R)-albuterol through the beta-2-adrenergic receptor. (24) Similarly, the *in vitro* guinea pig trachea preparation also showed that (R)-albuterol potently suppressed contractions produced by carbachol, histamine, and allergen (ovalbumin), whereas (S)-albuterol significantly increased the contractile response elicited by carbachol (25). Recent data reported by Delmotte and Sanderson (26) in mouse airways within lung slices, *in vitro*, have likewise demonstrated that (R)-albuterol decreases frequency of methacholine-induced intracellular Ca^{2+} oscillations and Ca^{2+} sensitivity, which was associated with increased relaxation, whereas (S)-albuterol did not demonstrate those properties. Thus, the theme of divergent effects of enantiomers of albuterol on airway contractility has been observed in a number of studies across species in airway models of study, *in vitro*. However, more evidence is necessary to support that (R)-albuterol within racemic albuterol is unequivocally adversely affected by (S)-albuterol, to the point that use of (R)-albuterol alone, should be universally applied in preference to racemic albuterol.

4.2.2. *In vivo* animal models of airway contractility

Animal studies *in vivo* have provided some additional data consistent with the *in vitro* findings mentioned above. For example, allergic bronchospasm in ovalbumin-sensitized guinea pigs was effectively abrogated by acute subcutaneous infusion of racemic albuterol (salbutamol; less than 1 hr.); however, longer infusion periods led to progressive susceptibility to spasmogens, and by 48 hr, ovalbumin exposure was fatal in most animals (27). The loss of protection at 48 hr was not due to a decrease in responsiveness of the beta-2-adrenergic receptor, as death could be prevented in animals pre-treated for 144 hr with racemic albuterol or isoproterenol. The negative effects might be attributable to (S)-albuterol, since a 1-hr (S)-albuterol infusion was associated with a significant increase in airway resistance. Those authors concluded that (R)-albuterol abrogates the bronchoconstrictive response during the initial treatment period (1–48 hr), but beyond that, opposing effects of (S)-albuterol predominated (27). Interestingly, but still remaining unexplained, the negative effects of (S)-albuterol could be prevented by sectioning the vagal nerve, again suggesting potential involvement of cholinergic pathways associated with (S)-albuterol effects. Further studies in ovalbumin-sensitized guinea pigs have shown that prolonged administration of both racemic albuterol and (S)-albuterol can induce bronchial responsiveness to histamine (28). Of note, both of those studies provided evidence in animals *in vivo* that racemic mixtures could eventually produce bronchoconstrictor effects that were similar to the paradoxical worsening of asthma reported in conjunction with use of racemic albuterol in humans, as mentioned above.

Similar to findings in the guinea pig models, a mouse model of daily inhaled racemic albuterol (0.2-20 micrograms) for 6 weeks demonstrated a significant albuterol time and concentration-dependent increase in bronchial hyperresponsiveness to methacholine, which was attenuated with inhaled budesonide, suggesting amelioration of the paradoxical chronic beta-2-adrenergic receptor agonist effects of racemic albuterol by steroid treatment (29). Furthermore, allergic airway inflammation models in mice have also shown differences in contractility with administration of albuterol enantiomers. One study showed that systemically-administered (S)-albuterol increased airway hyperresponsiveness, while (R)-albuterol, had little measurable effect. (30) However, there are no published studies of effects of inhaled beta-2-adrenergic receptor agonist enantiomers on airway contractility in mouse models of allergic airway inflammation, which should be undertaken in the future, to better understand the mechanisms of action of (S)-albuterol.

4.2.3. Human airway tissue contractility

A study of contractile responses of human bronchial tissues to spasmogens (methacholine, histamine, leukotriene C_4f, electric field stimulation (EFS), bradykinin, and others) were measured after incubation with (R)-albuterol, (S)-albuterol, and racemic albuterol ((R,S)-albuterol or salbutamol) *in vitro* (31). As would be expected, pre-incubation of tissues with (R)-albuterol reduced the sensitivity and response amplitude to the spasmogens methacholine and histamine. Interestingly, (S)-albuterol did not enhance tissue contraction by itself; rather, it magnified histamine, LTC_4 and IgE contractile responses. As with prior animal studies, the data suggested that (S)-albuterol may have acted through a non-beta-2-adrenergic receptor pathway; however, this potential pathway remains obscure and requires further study to more conclusively demonstrate its presence. Studies in cultured human ASM cells show that (S)-albuterol increases intracellular calcium levels in a dose-dependent fashion, and (R)-albuterol decreases them (32). These data agree with those obtained previously in bovine and guinea pig tracheal smooth muscle (22, 23, 25), and provide a potential mechanism through which (S)-albuterol may enhance airway smooth muscle contraction, which again contradicts its accepted status as an inert agent. Even so, it has also not been conclusively proven that (S)-albuterol within racemic albuterol exerts these effects uniformly, in these kinds of experiments.

4.3. Key preclinical enantiomer data on airway inflammation effects

In addition to inhibition of excitation/contraction coupling, beta-2-adrenergic receptor agonists can also reduce pro-inflammatory cytokine production, possibly via cAMP-mediated alterations in the activity of nuclear transcription factors such as IkappaB and NF-kappaB, which play a central role in regulating pro-inflammatory cytokine and chemokine transcription (33, 34). Signaling via the beta-2-adrenergic receptor may also alter glucocorticoid receptor complex formation that ultimately leads to upregulation of cell-cycle inhibitor protein $p21^{(Waf1/Cip1)}$ and decreased cell proliferation (35). These effects may underlie the synergy between corticosteroids and beta-2-adrenergic receptor agonists in the suppression of inflammation (36), and may account for the success of current beta-2-adrenergic receptor agonist and steroid combinatorial therapies in the treatment of asthma (36, 37). Because these mechanisms influence gene transcription, protein translation, and secretion, the onset of action of these beta-2-adrenergic receptor effects is much slower than the effects modulating contractility. While the majority of studies have been performed on racemic albuterol in a variety of cell models, at present, studies of albuterol enantiomers have just begun to delve into singular and combined effects on inflammation pathways at the molecular level.

4.3.1. *In vitro* leukocyte models of inflammation

While not universal, a number of studies of leukocytes *in vitro* support the postulate that (R)- and (S)-albuterol have opposing actions in the modulation of airway inflammation. The effects of albuterol enantiomers have been studied in a number of airway and immune cell types expressing beta-2-adrenergic receptors, including T lymphocytes, eosinophils, and mast cells, from both human and animal sources. For example, airway mast cells are considered to be key cell response elements in the allergic cascade. Mouse mast cells stimulated with IgE were unaffected by (R)-albuterol; however (S)-albuterol increased histamine release, and enhanced mRNA and protein production of IL-4 (38). The eosinophil represents another important immune effector cell in the allergic inflammatory process; secretion of eosinophil peroxidase was inhibited by both (R)-albuterol and racemic albuterol in eosinophils from allergic and non-allergic patients, but no effect of (S)-albuterol was observed (39). A subsequent pilot study suggested that both (R)-albuterol and racemic albuterol inhibited superoxide production of IL-5-activated human eosinophils, whereas (S)-albuterol increased superoxide production (40). At a minimum, the results suggest that (R)-albuterol can be considered anti-inflammatory where beta-2-adrenergic receptor-dependent eosinophil function is concerned. Thymus-derived lymphocytes (T-cells), particularly those of the Th2 subtype, are also important pathogenic components in allergic asthma. Studies in a mouse T-cell line have shown that (R)-albuterol can decrease IL-2 and IL-13 mRNA to a degree not observed of (S)-albuterol, and while (R)-albuterol did reduce IL-2 and IL-13 protein production, (S)-albuterol effects were similar (41). However, a comprehensive study of the effects of enantiomers on human T-cells demonstrated striking differences in (R)- and (S)-albuterol-mediated responses in human T-lymphocytes (42). In that study, (R)-albuterol inhibited T-cell proliferation, while (S)-albuterol had no effect; however addition of (S)-albuterol diminished the effects of (R)-albuterol in a concentration-dependent fashion. Additionally, the effects of both enantiomers were inhibited by propranolol, indicating that the observed effects were mediated through the beta-2-adrenergic receptor. Similar to the mouse T-cell study mentioned above (41), (R)-albuterol also decreased production of pro-inflammatory cytokines IL-2, IFN-gamma, IL-13, and IL-5 (42) . However, unlike the mouse T-cell studies, addition of (S)-albuterol to human T-cells *in vitro* resulted in significantly increased production of IL-2 and IL-13 (42), which has translational relevance toward the understanding of what enantiomers might do within humans. Thus, a number of studies of leukocytes have shown a both a strong anti-inflammatory effect of the (R)-albuterol enantiomer both individually and within the racemate, and a diminution of this effect by addition of the (S)-albuterol. Furthermore, several investigators have suggested that (S)-albuterol may act as an "inverse agonist," reversing or opposing the beta-2-adrenergic receptor agonist effects of (R)-albuterol (42-44), which would be counter to the accepted concept of (S)-albuterol as an inert enantiomer.

4.3.2. *In vitro* airway cell models of inflammation

Epithelial cells are important constitutive and functional cells within the airway that can influence airway caliber and secreted factor responses, as well as overall airway morphology. A study of racemic albuterol in human airway epithelial cell lines in serum-free culture, indicated that albuterol promoted growth equivalent to serum provision, acting similar to a growth factor (45). This effect of albuterol could be inhibited by propranolol and inhibition of cAMP, suggesting that the responses were associated with (R)-albuterol binding to the beta-2-adrenergic receptor, and may play a role in modifications of airway morphology associated with asthma and COPD. A subsequent study of albuterol enantiomers in primary human bronchial epithelial cells in culture indicated that (R)-albuterol significantly upregulated inducible nitric oxide synthase message in a concentration-dependent fashion, and downregulated GM-CSF message and protein release; (S)-albuterol was found to have none of these effects, again suggesting their specificity due to the (R) enantiomer (46). Thus, the above studies would suggest that albuterol effects in epithelial cells are mainly due to the (R)-enantiomer, either in the racemate, or alone, with no measurable effects of the (S)-enantiomer.

Similar to airway epithelial cells, airway smooth muscle (ASM) cells are also important constitutive and functional cells, which likewise can contribute to airway morphology as a result of growth and proliferation. However, unlike the case in airway epithelial cells, racemic albuterol has been reported to inhibit growth in human ASM cells (47, 48), and this effect appears to be dependent on time of exposure, showing no evidence of desensitization typically observed with prolonged and/or high concentrations of beta-2-adrenergic receptor agonists (49). While different than results in human airway epithelial cells, these effects in human ASM cells were inhibited with propranolol and ICI-118,551, and with cAMP antagonists

(47, 48), again suggesting effects dependent on binding to the beta-2-adrenergic receptor. However, the effects of individual albuterol enantiomers on proliferation have not been tested in human ASM cells; therefore, the question as to lack of effects of (S)-albuterol on proliferation remains unanswered, and ultimately may be different from that observed in airway epithelial cells.

ASM cells also have non-contractile paracrine-like functions, producing important cytokines including GM-CSF, IL-6, and IL-11 (50, 51), and may be important regulators of airway inflammation. Some basic studies in cell cultures have shown that racemic albuterol can downregulate ASM release of those important cytokines. Similarly, the individual (R)-albuterol enantiomer reduced GM-CSF production of cytokine stimulated human ASM cells, whereas (S)-albuterol increased its production (52). In parallel to the T-cell data, addition of (S)-albuterol with (R)-albuterol diminished the effect of (R)-albuterol, and the effects of (R)-albuterol were reversed by propranolol. Furthermore, (R)-albuterol amplified the anti-inflammatory effect of dexamethasone in reducing GM-CSF production, whereas (S)-albuterol blunted this effect. Most notably, both (S)-albuterol and propranolol increased GM-CSF production similar to ICI-118,551, a potent and maximal inverse agonist, suggesting inverse agonism as a potential mechanism of (S)-albuterol (53). These findings are of significant import because GM-CSF is considered a strong pro-inflammatory allergic cytokine due to its ability to promote eosinophil activation and survival (54, 55); thus, its modulation by (R)-enantiomers and (S)-enantiomers may be an important aspect of the control of inflammation by beta-2-adrenergic receptor agonists.

In this regard, an important study that has provided some clues regarding potential intracellular mechanisms of albuterol single enantiomer effects was reported by Agrawal, *et al.* (32). Prior studies have shown beta-2-adrenergic receptor agonist-induced elevations in cAMP produce reductions in GM-CSF release by human ASM cells (47, 56). Consistent with this notion, (R)-albuterol increased stimulatory G-protein (Gs) expression and activation with associated elevations in cAMP; however, (S)-albuterol reduced Gs expression and activation, and increased inhibitory G-protein (Gi) expression (32). In turn, these alterations in G-protein expression were found to be associated with reduced intracellular cAMP levels and increased inositol triphosphate (PI3) kinase and NF-kappaB activation (32). A subsequent study in human ASM cells has supported these findings, indicating that the cytokine suppressive effects of (R)-albuterol in ASM can occur through PI3-kinase-dependent decreased phosphorylation of IκappaBalpha, in turn, decreasing NF-kappaB release from the IkappaB/NF-kappaB complex. (57) Given these data, it would appear that ASM cells may be important contributors to the airway inflammatory response, and that enantiomers of beta-2-adrenergic receptor agonists have measurable differences in their effects on those cells, which may down-regulate or up-regulate the inflammatory process in the airway.

4.3.3. *In vivo* animal studies of airway inflammation

A mouse model of daily inhaled racemic albuterol (0.2-20 micrograms) for 6 weeks demonstrated a significant albuterol time and concentration-dependent increase in airway mucosal epithelial cell proliferation and wall thickening, which was attenuated with inhaled budesonide, suggesting amelioration of the paradoxical chronic beta-2-adrenergic receptor agonist effects of racemic albuterol by steroid treatment (29). Those effects on airway wall morphology were also inhibited by administration of MAPkinase inhibitors, suggesting a potential mechanism for their appearance due to albuterol, as well as how they may be mitigated by steroid treatment (29). Those findings are in agreement with prior findings with racemic albuterol administration to human airway epithelial cell lines, *in vitro* (45), as mentioned above, suggesting consistency of this effect mechanism at different levels of integration from cell cultures to whole animals.

While there have been a number of studies of effects of racemic albuterol in animals, there have been few published studies specifically focusing on the effects of beta-2-adrenergic receptor agonist enantiomers on airway inflammation in animals. A subsequent study of systemically-administered albuterol enantiomers from peripherally-implanted mini-osmotic pumps in ovalbumin-treated mice with allergic airway inflammation indicated that (R)-albuterol decreased airway and parenchymal eosinophilia, goblet cell hyperplasia, BAL IL-4 levels, and plasma IgE (30). Although (S)-albuterol, in that same study, was reported to increase lung tissue edema and airway hyperresponsiveness, it was also reported to have similar effects on those variables mentioned above (30), which was puzzling, given data reported in other studies outlined above, and below. A subsequent study of slowly-released albuterol enantiomers from peripherally-implanted mini-osmotic pumps in ovalbumin-treated mice with allergic airway inflammation likewise suggested that (R)-albuterol reduced histological evidence of airway inflammation; however, decreases in BAL eosinophils and serum IgE were not statistically significant (41).

Based on *in vitro* data outlined in prior subsections above, some of the unexpected similarity of effects of beta-2-adrenergic receptor agonist enantiomers in animal models is likely due to confounding effects related to peripheral administration of the tested drugs, and argues for investigations with direct aerosolized administration of inhaled single enantiomers. In this instance, there is only one published study of inhaled aerosolized beta-2-adrenergic receptor agonist enantiomers with airway inflammation in rats, but it must be considered with the caveat that the model was one of respiratory syncycial virus (RSV)-associated infection, as opposed to allergic airway inflammation (58). In that study, (R)-albuterol at clinically relevant doses (0.31-1.25 mg) was shown to decrease and ultimately abolish neurogenic extravasation associated with viral inflammation (58). Conversely, (S)-albuterol had no significant inhibitory effect up to 1.25 mg, and racemic albuterol demonstrated a blunted inhibitory effect as compared to (R)-albuterol, alone (58). Data from this solitary study of inhaled agonists in animals is consistent with expectations of a divergence of effects between the enantiomers, and supports that further study of inhaled beta-2-adrenergic receptor agonist enantiomers are needed to close the loop in our understanding of how these drugs work differently in the

setting of allergic airway inflammation. Until those studies are performed, there remains no overwhelming evidence of albuterol enantiomer superiority over the racemate in those animal models.

In summary, the available pre-clinical data mainly, although not universally, indicate opposing effects of the enantiomers of albuterol on contractile and inflammatory mechanisms in human and animal airway cell systems and tissues, and within animals, *in vivo*. The desirable effects attributed to beta-2-adrenergic receptor agonists, such as airway smooth muscle relaxation and inhibition of inflammation, are consistent with the action of the (R)-enantiomer of albuterol in both single enantiomer and racemic formulations. Although having 100-fold less binding affinity for the beta-2-adrenergic receptor, the pre-clinical data generally indicate that (S)-albuterol is neither inactive nor inert, and in fact, has effects counter to those of the (R)-albuterol. The pro-inflammatory effects of (S)-albuterol are variable in magnitude, and in some cases not significant, particularly in the presence of high-affinity agonist (R)-albuterol. However, as shown in Figure 1, the pharmacokinetic profile of the two enantiomers, with rapid metabolism of (R)-albuterol, slower metabolism of (S)-albuterol, and subsequent persistence of (S)-albuterol (59), suggests that (S)-albuterol effects must be accounted for in any *in vivo* model of study that utilizes racemic albuterol. With these effects in mind, a brief review of relevant clinical studies conducted with enantiomers and racemates of albuterol, follows below.

5. BRONCHOPROVOCATION STUDIES OF ENANTIOMERS IN ASTHMATICS

Several small studies of the effects of albuterol enantiomers on methacholine bronchoprovocation in asthmatics (n less than 33 patients per study) have been conducted within the past 15 years. Initial interest in these effects was sparked by the study of Perrine-Feyolle and colleagues (60), in which (R)-albuterol was found to suppress methacholine-induced bronchoconstriction more effectively than racemic albuterol and (S)-albuterol. In contrast, subsequent double-blind, randomized cross-over studies by Cockcroft and colleagues indicated that 1.25 mg of (R)-albuterol was equivalent to 2.5 mg racemic albuterol in bronchodilatory effects, whereas (S)-albuterol had little or no effect (61, 62). Those results were corroborated by Ramsay, *et al.*, (63) with administration of even smaller drug concentrations (0.1-0.2 mg). A recent double-blind, randomized cross-over study in twenty-six mild to moderate asthmatics by Sjosward, *et al.*, (64), has reported no favorable effects of (R)-albuterol over the racemate, when tested using isocapnic hyperventilation of cold air as a bronchoprovocation. However, it is important to note that many of the results in the above studies were typically short-term assessments (less than or equal to 3 hours over 1-7 days) obtained after a single administration of the respective drugs, which would be expected to show a strong effect of both (R)-albuterol and racemic albuterol (due to the presence of (R)-albuterol), and no real effect of (S)-albuterol, based on the known significant differences in beta-2-adrenergic receptor affinity between (R)- and (S)-albuterol. Thus, while demonstrating an expected and desirable effect due to the presence of (R)-albuterol, the design of those studies makes difficult a comprehensive assessment of differences between (R)- and (S)-albuterol with chronic use, which require additional administrations and longer intervals of study, based on the important differences in pharmacokinetic characteristics of (R)- and (S)-albuterol, outlined above.

6. CLINICAL EFFECTS POTENTIALLY ASSOCIATED WITH (S)-ALBUTEROL

One important aspect potentially contributing to the paradoxical worsening of asthma may be the use of racemic albuterol as a chronic "reliever" medicine, as opposed to a treatment to counter an acute exacerbation, particularly in the setting of inhaled corticosteroid (ICS) non-compliance. One would suppose that patients utilizing racemic albuterol in this fashion would be more likely to be classified as moderate to severe, rather than mild, asthmatics. However, as pointed out by Wraight, *et al.*, (65) patients with unstable asthma, who might be classified as something other than mild, and who would be most likely to use racemic albuterol more often as a "reliever," were excluded from many prior studies (66-69). The number of patients to whom this might apply has been reported by Tattersfield, *et al.* (70) to be as high as 25-30% of all asthmatics. Accordingly, this scenario cannot be considered uncommon, and bears further examination, particularly from the perspectives that: 1) severe asthmatics have been reported to have 2-5-fold higher levels of plasma (S)-albuterol (71), and 2) non-steroid-treated asthmatics appear to have high serum levels of (S)-albuterol, as compared to steroid-treated asthmatics and healthy volunteers (72, 73).

A recent clinical trial evaluating the effects of chronically-administered short-acting beta-2-adrenergic receptor agonists supports the notion of chronic racemic albuterol use leading to paradoxical worsening of asthma, in the setting of ICS withdrawal (65). These data demonstrated that adverse changes in lung function with ICS withdrawal were exacerbated, occurring more rapidly in asthmatic subjects on racemic albuterol than in those on racemic albuterol plus ipratropium. Based on ASM cell culture experiments *in vitro* (74), a potential explanation offered for the results was inflammatory cytokine-associated desensitization of the beta-2-adrenergic receptor. However, another potential explanation is that the use of racemic salbutamol led to adverse changes in lung function and inflammation through the effects of (S)-albuterol (75), unavoidably contained within the racemate (76). Pre-clinical data outlined above, in both humans and animals, suggest that these alterations in lung function might be due to (S)-albuterol-mediated elevations in intracellular calcium in ASM and airway narrowing (23, 27, 31, 32). This may be an example of steroid suppression of (S)-albuterol effects that are unmasked when ICS is withdrawn, and (S)-albuterol persists (59), potentially influencing airway responsiveness. Conversely, these results also suggest another potential reason behind the lack of differentiation between (R)-albuterol and the racemate, in studies in which inhaled steroid therapy is maintained during investigation of airway reactivity (64)

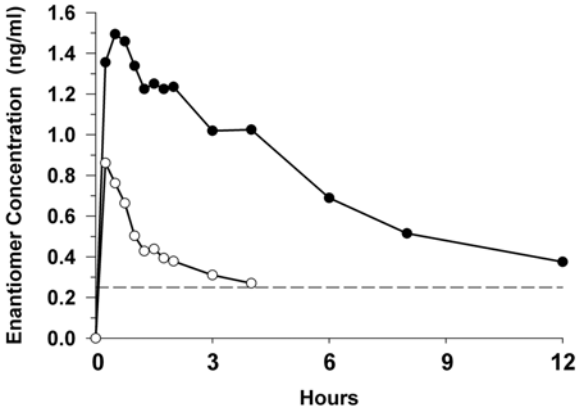

Figure 1. Mean human plasma concentrations of (R)-albuterol, as open circles (○), and (S)-albuterol, as filled circles (●), as a function of time, after a single inhalation of racemic albuterol (2.5 mg). Dashed horizontal line indicates limit of assay detection. Note that circulating (S)-albuterol concentrations persist more than 9 hours after (R)-albuterol concentrations have become undetectable. Reproduced with permission from ref. (59).

Finally, a recent study performed in otherwise-healthy volunteers with a history of exercise-induced bronchospasm indicated that regular, twice-daily use of racemic salbutamol for one week not only led to increased exercise-induced bronchoconstriction, but also a failure to bronchodilate completely, in response to acutely administered racemic albuterol during exercise-induced bronchoconstriction (77). Similar to Wraight, *et al.*, (65) those authors suggested desensitization of the beta-2-adrenergic receptor as one potential explanation, and possibly increased airway inflammation as another, based on studies showing increased sputum eosinophils associated with regular short-acting beta-agonist use (78, 79). There also remains the distinct possibility that accumulation of (S)-albuterol from the racemic mixture contributed to the observed bronchoconstrictive effects. Thus, in light of the pre-clinical and clinical data presented above, the available data of larger, more comprehensive clinical trials of albuterol enantiomers are of interest, and are presented below.

7. EFFECTS OF ALBUTEROL IN CLINICAL TRIALS

7.1. Randomized controlled efficacy clinical trials of albuterol in asthma

The earliest published controlled trial comparing various doses of racemic albuterol and (R)-albuterol was published by Nelson and colleagues (80). More than 350 adolescent and adult patients were randomized to 0.63 mg (R)-albuterol, 1.25 mg (R)-albuterol, 1.25 mg racemic albuterol, or 2.5 mg racemic albuterol (80). These doses provided two levels of equivalent mass of (R)-albuterol, with or without the (S)-albuterol component. At first dosing, but not after 4 weeks of thrice daily therapy, there were significantly greater improvements in FEV_1 in the levalbuterol groups compared to the dose-equivalent racemic groups. The dose providing numerically equivalent bronchodilation to 2.5 mg racemic albuterol was 0.63 mg of (R)-albuterol, not 1.25 mg (R)-albuterol (the mass equivalent dose). These data have been interpreted as showing a potential detrimental effect of (S)-albuterol, but those suggestions have been challenged (81). Interestingly, a recent post-hoc analysis of those data have further supported that (R)-albuterol provided greater bronchodilation than the same quantity of (R)-albuterol contained in the racemate, which is again suggestive, but does not prove, that (S)-albuterol within the racemate may compromise the effects of (R)-albuterol, in some asthmatics (82).

Pediatric patients have also been studied for responses to differing albuterol formulations. Gawchik and colleagues evaluated 43 children treated with (R)-albuterol compared to racemic albuterol in a randomized, placebo-controlled comparator trial. No significant differences were observed in FEV_1 response among the active treatment groups. However, it is important to note that this study was powered for efficacy versus placebo, and was not powered to discriminate among the treatment groups (83).

Milgrom and colleagues evaluated 338 pediatric patients in a double-blinded, placebo controlled, randomized trial of (R)-albuterol (0.31 mg and 0.63 mg, 3 times per day) versus racemic albuterol (1.25 mg and 2.5 mg, 3 times per day), using a primary endpoint of change in FEV_1 (84). Equivalent bronchodilation was observed in all arms: the authors could not demonstrate a dose-response; however, safety indices were not different from placebo only in the (R)-albuterol 0.31 mg arm. On the basis of these data, the authors suggested that there was a safety advantage to the use of 0.31 mg (R)-albuterol as a starting dose in children. In children with more severe asthma, a dose-relationship was observed for (R)-albuterol only, suggesting that in more severe disease, increased dosing might be useful. The limitation of this study is that dose-dependency of the primary outcome variable, change in FEV_1, could not be established in the entire study population. Accordingly, the concerns advanced by Aherns and Weinberger (81) about the Nelson study (80), are applicable to this study as well.

Lotvall and colleagues conducted a double-blinded, crossover study of the comparative efficacy of racemic albuterol and (R)-albuterol, using a design developed to assess the therapeutic ratio of these compounds. Measures of efficacy included FEV_1 improvement, and safety markers included change in heart rate and serum potassium concentration (85). On a mg/mg basis, (R)-albuterol was twice as effective as racemic albuterol (the expected difference, based on the mass of available active levalbuterol) for both efficacy and safety measures. In those studies, (S)-albuterol and placebo were equivalent, and (R)-albuterol and racemic albuterol were equivalent. Consequently, the authors concluded that there was no increase in therapeutic index for the single enantiomer, as compared to the racemate. These data were accompanied by an editorial that argued against any compelling reason to utilize (R)-albuterol over the less expensive racemic alternative (81).

7.2. Controlled trials in acute asthma in adults and children

Using a randomized, double-blinded, age stratified (less than 6, or 6-18 yrs) trial design, Carl and colleagues compared (R)-albuterol to racemic albuterol by aerosol in 547 patients presenting to the emergency department (ED) with asthma. Management in the ED was conducted according to a standardized asthma care algorithm with 1.25 mg (R)-albuterol *vs.* 2.5 mg racemic albuterol (1.25 mg (R)-albuterol plus 1.25 mg (S)-albuterol (86) delivered every 20 minutes for a maximum of 2 hours; those patients who did not meet defined objective criteria for discharge were admitted to the hospital. The primary outcome measure was hospital admission rate. Analysis of the randomized population demonstrated no significant differences in demographic or clinical characteristics. African-Americans comprised more than 80% of the enrollees, and 2/3 were male. Use of inhaled steroids (less than 50%), oral steroids within 24 hours (approximately 6%), leukotriene modifiers (less than 33%), and long acting beta-2-adrenergic receptor agonists (approximately 15%) was similar between groups; significantly more patients randomized to (R)-albuterol were using cromolyn (68% *vs.* 42%). Patients treated with (R)-albuterol had significantly fewer hospitalizations compared to those treated with racemic drug (35% *vs*. 45%). Moreover, in patients who had received more than 3 aerosol albuterol treatments and had received oral corticosteroids, the relative risk of hospital admission was 1.34 in patients treated with racemic albuterol compared to (R)-albuterol, suggesting that patients presenting with more severe asthma also may benefit from (R)-albuterol therapy for acute exacerbations.

In support of this concept was an ED study by Nowak and colleagues (17), which had some focus on measurement of (S)-albuterol levels. Plasma (S)-albuterol levels were inversely correlated with pulmonary function on admission to the ED (FEV_1), and were also significantly negatively correlated with response to bronchodilator administration in the ED. Further recent pilot data have been consistent with those findings, suggesting that patients with high plasma levels of (S)-albuterol have slower improvement in FEV_1 and a greater likelihood of hospital admission (87), and, that patients given (R)-albuterol have more rapid improvement in FEV_1 as compared with those given racemic albuterol (87). While these findings require confirmation in a full study, they are consonant with prior clinical studies, and expectations based on prior basic studies, outlined above.

However, there have also been several recent pediatric asthma ED clinical studies involving 70-140 patients, that have reported no difference between racemic albuterol and (R)-albuterol for either primary outcome measures (FEV_1, oxygen saturations, respiratory rates, peak flow rates, and length of ED stay), or secondary outcome measures (number of treatments, length of ED stay, and hospital admission rates) (88-90). It is important to note that those clinical studies acknowledged limitations of small study size (88) and lack of reliability of pulmonary function tests collected in children (90). Protocol-associated drug administration confounds within these trials were also present as: 1) administration of additional steroids and ipratropium during albuterol treatment (88, 90), 2) administration of racemic albuterol at 4x the dose of (R)-albuterol (5 mg racemic albuterol vs. 1.25 mg (R)-albuterol) (89), 3) administration of ipratropium only to the racemic albuterol group (89) and not the single enantiomer treatment group, and 4) exclusion of subjects from study that had taken (R)-albuterol previously, but not those who had taken racemic albuterol, previously (90). It is not clear why these confounding characteristics were present, but their presence makes it difficult to evaluate the meaning of the results of those studies in the context of comparisons of single enantiomer efficacy, as compared with the racemic version. Even so, most agree that the results suggest that further comparative studies are necessary to resolve the true properties of these compounds in asthmatics with more certitude.

8. SUMMARY

Considerable pre-clinical data strongly suggest that (R)- and (S)-enantiomers of albuterol are not equivalent, in that: 1) (R)-albuterol contains essentially all of the beta-2-receptor agonist activity, and consequently is responsible for the bronchodilation and modulation of inflammatory activity that is attributable to beta-2-receptor agonists, 2) (S)-albuterol administered by itself is generally pro-inflammatory in many instances tested, 3) (S)-albuterol in acute combination with (R)-albuterol generally abrogates the anti-inflammatory activity of (R)-albuterol, and 4) (S)-albuterol is metabolized considerably more slowly that (R)-albuterol, with the consequent possibility of accumulation of (S)-albuterol with repeated use, and unopposed (S)-albuterol effects with acute dosing after (R)-albuterol has been metabolized.

Consistent with the pre-clinical data are several randomized controlled clinical trials and post-hoc analyses showing both numerical and statistical superiority of (R)-albuterol over racemic albuterol. However, in most of those trials, which were not powered to demonstrate statistically significant differences between active treatments (91), strict dose dependency of the outcome variable was not achieved, and so the question of pharmacologic superiority of (R)-albuterol over racemic albuterol remains controversial, and formally unanswered. Now that an (R)-albuterol metered-dose inhaler is available, it would be possible to answer that question in a direct head-to-head fashion that would be convenient for study participants, as well as investigators. However, at this time, it remains unclear as to whether this study will be undertaken, in order to finally resolve this question of single enantiomer (R)-albuterol superiority over racemic albuterol.

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Abbreviations: ASM: airway smooth muscle; BAL: bronchoalveolar lavage; Ca^{2+}: calcium ion; cAMP: cyclic adenosine monophosphate; CFC: chlorofluorocarbon; COPD: chronic obstructive pulmonary disease; D-: dextrorotatory; ED: emergency department; EFS: electric field stimulation; FEV_1: forced expiratory volume, 1st second; GM-CSF: granulocyte-macrophage colony stimulating factor; GPCR: G-protein coupled receptor; HFA: hydrofluoroalkane; HPLC: high performance liquid column chromatography; ICS: inhaled corticosteroids; IFN: interferon; i.p.: intraperitoneal; L-: levorotatory; LTC: leukotriene C; MDI: metered-dose inhaler; mRNA: messenger ribonucleic acid; PI3: inositol-1,4,5-triphosphate; (R): rectus; RSV: respiratory syncycial virus; (S): sinister

Key words: Airway smooth muscle, Beta-2-receptor agonists, Bronchoconstriction, Racemic albuterol, (R)-albuterol, (S)-Albuterol, Review

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