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Short communication

Salmeterol undergoes enantioselective bronchopulmonary distribution with receptor localisation a likely determinant of duration of action

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ABSTRACT

Background: Salmeterol (a long acting beta2-agonist) is a chiral molecule. (R*R*)-salmeterol is responsible for pharmacological effect, but basic knowledge of enantioselective pulmonary pharmacodynamics and pharmacokinetics of salmeterol remains unknown. There are safety concerns with (*S*)-enantiomers of beta2-agonists, with suggestions that these enantiomers may increase bronchial hyperresponsivneness in asthma patients.

Methodology: Horses (n = 12) received racemic (*rac*-) salmeterol 250 μg via inhalation. Enantioselective UPLC–MS/MS was used to determine (*R*)- and (*S*)-salmeterol concentrations in pulmonary epithelial lining fluid (PELF) sampled 2, 5, 10 and 15 min after administration, in central lung (endoscopic bronchial biopsy) and peripheral lung (percutaneous pulmonary biopsy) tissues (at 20 and 25 min respectively), and in plasma samples.

Results: Physiologically relevant tissue concentrations were found for both enantiomers, with median levels greater in central than peripheral lung (equivalent to 32 and 5 mM (*R*)-salmeterol for central and peripheral lung respectively). Levels in PELF decreased around 50% over 15 min and enantioselective distribution was observed in the central lung with levels of (*R*)-salmeterol around 30% higher than (*S*)-salmeterol.

Conclusion: Salmeterol distribution is enantioselective in the central lung. This suggests duration of action is more likely associated with specific B2ADR localisation effects rather than non-specific physiochemical factors which would not be enantioselective.

1. Introduction

Salmeterol, a long acting beta2-agonist (LABA) is a chiral molecule administered as a 50:50 racemic (*rac*-) mixture of two non-superimposable mirror image molecules called enantiomers ((*R*)-salmeterol and (*S*)-salmeterol; Fig. 1). For salmeterol, beta2-adrenoceptor (B2ADR) pharmacological activity resides in (*R*)-salmeterol, while (*S*)-salmeterol is generally considered to be inert at this target. The difference in B2ADR activity between (*R*)- and (*S*)-salmeterol has been reported to be approximately 40-fold [1], less than for other beta2-agonists. The most basic knowledge regarding pharmacodynamics, drug disposition and fate of (*S*)-enantiomers in the lung, has remained elusive for over 20 years. This is despite FDA expectations that new chiral drug applications are enantiopure unless there is evidence that the racemic mixture is safe and recommendations that enantioselective assays be developed for use early in drug discovery for assessment of pharmacokinetics [2].

The chiral nature of these compounds has been largely forgotten in the debate around LABA safety, and enantioselectivity of LABA pharmacodynamics could be an important determinant of adverse effects in some individuals [3]. Rare but serious, and as yet unexplained, paradoxical asthma exacerbations have been associated with LABAs, and chronic use is associated with increased bronchial hyperresponsiveness (BHR) and loss of bronchoprotection [4]. While little is known about the adverse pharmacodynamics of (*S*)-salmeterol, there are convincing *in vitro* studies showing that (*S*)- enantiomers of short acting beta2-agonists are associated with

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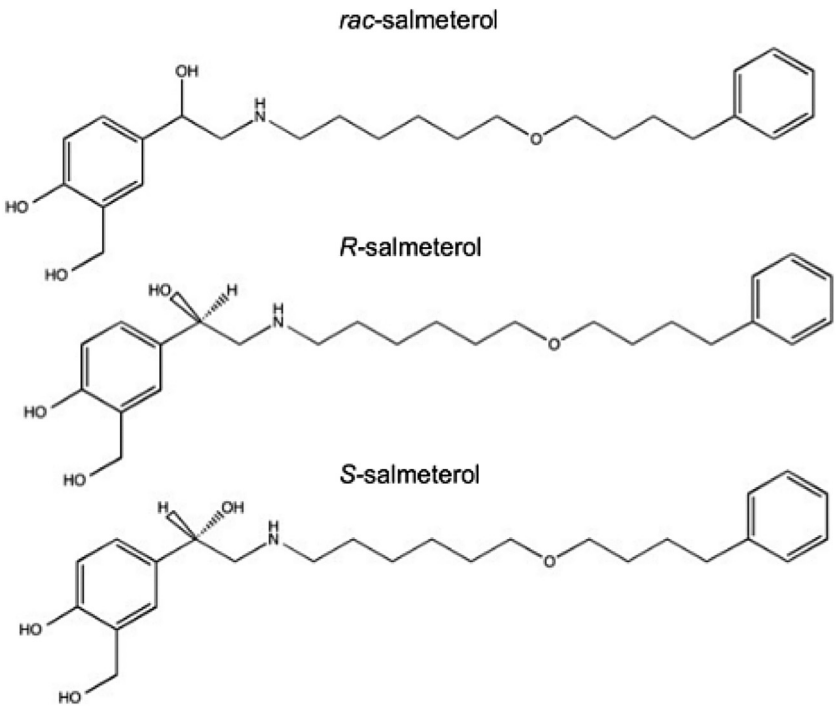

Fig. 1. Structure of salmeterol enantiomers.

a range of adverse airways effects including enhanced contraction of isolated human bronchial tissue [5] and increased airway responsiveness [6].

Rac-salmeterol does appear to be enantioselective in terms of pharmacodynamics, but interestingly not to the same extent as other beta2-agonists [7]. Furthermore, the mechanisms behind sustained activity at the B2ADR are still not fully understood, with duration either due to physicochemical interactions with lipid bilayers, or more specific B2ADR exosite localisation [8]. Unlike physicochemical processes such as lipid partitioning, individual enantiomers in living biological systems can differ significantly in specific structure-activity relationships involving transporters, drug receptors and metabolism enzymes [8]. It follows, therefore, that significant differences in lung tissue concentration between salmeterol enantiomers would be suggestive of enantioselective mechanisms in lung pharmacokinetics rather than physicochemical factors such as lipid partitioning. Investigations into human organic cation transport (OCT) proteins, which are thought to be involved in beta2-agonist transport in the lungs, have suggested stereoselective binding with drugs closely related to salmeterol [9,10]. Furthermore, it is apparent that concomitant corticosteroid use may interfere with removal of cationic drugs by OCT in the airway with the effect more apparent with formoterol compared to salmeterol [11].

The objective of this study was to measure salmeterol enantiomers in pulmonary epithelial lining fluid (PELF) together with the central and peripheral lung tissue of horses, to determine if there was enantioselective disposition after administration of a single dose of *rac*-salmeterol via inhalation, and to better understand the fate of (*S*)-salmeterol in the lung. Horses are one of few species that spontaneously develop reversible airway obstruction similar to asthma [12,13]. Horses are also well suited to airway sampling due to their large size and poorly developed airway protective reflexes, allowing direct sampling of PELF and bronchial epithelial biopsy from their conducting airways at low welfare cost. Percutaneous lung biopsy is also well tolerated and used clinically with relative safety to the animal.

2. Materials and methods

2.1. Equine drug administration

The study protocol was approved by the Animal Care and Ethics Committee (Charles Sturt University) ACEC 15/039; horses were recruited via convenience sampling from a research herd kept outdoors in paddocks, with demographics as outlined previously [14]. Each underwent a veterinary physical examination prior to receiving a single dose of inhaled *rac*-salmeterol (Seretide®, salmeterol 25 μg and fluticasone 50 μg per metered dose, GlaxoSmithKline Pty Ltd, Victoria, Australia) of 250 μg delivered by inhalation. For each horse, a total of 10 actuations ('puffs') was administered via a spacer whilst the inhalation device (AeroHippus, Trudell Medical International, Ontario, Canada) was held over the horse's nostril, with the release of medication timed to coincide with inhalation.

2.2. Lung and blood sampling

PELF and lung tissue sampling was performed as previously outlined using cotton tip swabs sheathed in PVC tubing [14]. Timed PELF collection was performed at 2, 5, 10 and 15 min following administration of the full inhalation dose. The cotton head of each PELF swab was cut from its stick and placed in an Eppendorf® centrifuge tube and weighed. After the addition of 10 ng *rac*-salmeterol-d3 in 10 μL as internal standard (Toronto Research Chemicals, Toronto, Canada), the swab was extracted with 1000 μL methanol by soaking overnight and vortex mixing, followed by a repeat extraction of 500 μL. The methanol extracts were combined and transferred to a glass vial, and reduced to dryness under nitrogen at 40 °C. An additional step to our previous work [7] was introduced to improve recovery, presumably due to pH or buffer capacity effects, whereby samples were reconstituted in 400 μL water adjusted to pH 8.5 with ammonium hydroxide, evaporated under nitrogen at 40 °C, and reconstituted with 80 μL of methanol.

Salmeterol enantiomer levels were quantified by ratio of each enantiomer analyte to deuterated internal standard. The extracted