Effects Of β_2-adrenoceptor Agonists On Cytokine Generation From Human Lung Macrophages

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Lung macrophages are believed to be involved in the pathogenesis of Chronic Obstructive Pulmonary Disease (COPD) and may also have a role in asthma (Barnes, 2008). These inflammatory lung diseases are exacerbated by infections. Currently, treatments for COPD and asthma include steroids such as Dexamethasone as anti-inflammatory agents. β_2-adrenoceptor agonists are also reported to have anti-inflammatory effects but this is disputed (Donnelly *et al.*, 2010; Ezeamuzie *et al.*, 2010).

The aim of this study was to investigate the inhibitory effects of long and short-acting β_2-agonists on pro-inflammatory cytokine generation from human lung macrophages in conditions simulating infection (Lipopolysaccharide (LPS)).

Macrophages were isolated from macroscopically normal resected lung tissue of adult participants undergoing surgery mainly for lung carcinoma. Ethical approval and informed written consent were obtained. Cells were purified by Percoll density gradient centrifugation (mean purity $91\pm2\%$ macrophages, n=6) according to modification of the protocol from Liu *et al.* (1984). The cells were incubated overnight, in 24-well culture plates at 2×10^5 cells/well before challenge with LPS (10 ng/ml) in the presence or absence of short-acting β_2-agonists (Isoprenaline, Terbutaline, Salbutamol) (all 10^{-5} M), long-acting β_2-agonists (Formoterol, Salmeterol, Indacaterol) (all 10^{-5} M) or the steroid Dexamethasone (10^{-7} M). Cell culture supernatants were harvested at 22 h and assayed for TNF-α, IL-6, and IL-8 by ELISA. Short-acting β_2-agonists were prepared as stocks (10^{-2} M) in aqueous solution. Long-acting β_2-agonists (10^{-2} M) and Dexamethasone (10^{-1} M) were prepared as stocks in Dimethyl Sulphoxide. Statistical significance was determined by 1-way ANOVA followed by Dunnett's Multiple Comparison test. Significant values were defined as $p<0.05$.

Challenge of macrophages with LPS resulted in high levels of pro-inflammatory cytokine release. Short-acting β_2-agonists showed no significant inhibitory effects on LPS-stimulated cytokine generation. However, the level of TNF-α release ($1,108\pm271$ pg/ml) was significantly decreased by Salmeterol (570 ± 146 pg/ml, $p<0.01$), Indacaterol (712 ± 205 pg/ml, $p<0.05$) and the steroid Dexamethasone (171 ± 41 pg/ml, $p<0.001$) (n=6). Inhibition of IL-6 generation followed a similar pattern. The level of IL-6 release ($4,896\pm1,478$ pg/ml) was decreased by Salmeterol (2159 ± 691 pg/ml, $p<0.01$), Indacaterol (2686 ± 881 pg/ml, $p<0.05$) and Dexamethasone (1007 ± 470 pg/ml, $p<0.001$) (n=5). The only compound found to significantly inhibit IL-8 generation ($55,414\pm13,729$ pg/ml) was Dexamethasone ($12,181\pm3,835$, $p<0.001$) (n=5).

In summary, macrophages isolated from human lung tissue respond to LPS challenge with the release of high levels of pro-inflammatory cytokines. TNF-α and IL-6 generation is effectively inhibited by some long-acting β_2-agonists (Salmeterol and Indacaterol) but not short-acting β_2-agonists. IL-8 generation is not inhibited by any of the β_2-agonists investigated. To conclude, the data show that β_2-agonists have differential effects on LPS-induced cytokine generation from human lung macrophages.

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