Effect of salmeterol/fluticasone propionate on airway inflammation in COPD: a randomised controlled trial

Jean Bourbeau, Pota Christodouloupolous, Francois Maltais, Yasuhiro Yamauchi, Ronald Olivenstein, Qutayba Hamid

Background: Airway inflammation in chronic obstructive pulmonary disease (COPD) is characterised by infiltration of CD8+ T cells and CD68+ macrophages and an increased number of neutrophils, whereas few studies have described the presence of eosinophils. Although the anti-inflammatory effects of corticosteroids in stable COPD are unclear, recent studies suggest that combination therapy could be beneficial. A study was therefore undertaken to evaluate combined salmeterol/fluticasone propionate (SFC) and fluticasone propionate (FP) alone on inflammatory cells in the airways of patients with COPD.

Methods: Patients were treated in a randomised, double blind, parallel group, placebo-controlled trial with either a combination of 50 μg salmeterol and 500 μg FP twice daily (SFC, n = 19, 19 men, mean age 62 years), 500 μg FP twice daily (n = 20, 15 men, mean age 64 years) or placebo (n = 21, 17 men, mean age 66 years) for 3 months. At the start and end of treatment bronchoscopy with bronchial biopsies was performed and the numbers of CD8+ T lymphocytes, CD68+ macrophages, neutrophils and eosinophils were measured.

Results: CD8+ cells were significantly reduced by SFC compared with placebo (difference −98.05 cells/mm²; 95% CI −143.14 to −52.9; p < 0.001). Such a marked effect was not seen with FP alone (−44.67 cells/mm²; 95% CI −90.92 to 1.57; p = 0.06). CD68+ macrophages were also reduced by SFC compared with placebo (difference −31.68 cells/mm²; 95% CI −61.07 to −2.29; p = 0.03) but not by FP. SFC did not significantly change neutrophils and eosinophils compared with placebo.

Conclusions: SFC has airway anti-inflammatory effects not seen with inhaled corticosteroids alone.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE

C hronic obstructive pulmonary disease (COPD) is a multi-component disease characterised by progressive airflow limitation that is not fully reversible. Central to its pathogenesis is a chronic inflammatory process throughout the airways, parenchyma and pulmonary vasculature resulting in many structural changes. The predominant pattern of airway inflammation in COPD is a prevalence of T lymphocytes and macrophages which have been shown to correlate with disease severity. T lymphocytes, with a greater increase in CD8+ than in CD4+ T cells, have been shown to relate to lung destruction and inversely to decline in lung function. CD68+ macrophages appear to play a pivotal role in COPD. A correlation has been demonstrated between macrophages in the airways and parenchymal destruction, chronic exposure to cigarette smoke and COPD severity. Neutrophils are also increased within COPD airways, although their role is not completely understood. They have been associated with COPD disease severity and with the rate of decline in lung function, as they have the capacity to induce tissue damage through the release of various oxidants and proteases. Increased numbers of eosinophils have also been reported but their role in COPD remains questionable.

Although there is evidence that inhaled corticosteroids reduce COPD exacerbations, their beneficial effects on airway inflammation in stable COPD appear limited. However, a combination of long-acting β2 agonist and inhaled corticosteroids not only reduces exacerbations in COPD but improves lung function and symptom control to a greater extent than either component alone. Recently, combination therapy has been shown to reduce airway inflammation in COPD.

To date, however, there has been no comprehensive study examining differences in the effects of combination therapy and inhaled corticosteroids alone on the inflammatory profile in COPD airways. We postulated that a combination of a long-acting β2 agonist with an inhaled corticosteroid—and, to a lesser extent, inhaled corticosteroid alone—would reduce airway inflammation, specifically CD8+ T cells and CD68+ macrophages in COPD after 3 months of treatment. Furthermore, as a secondary outcome in this study, we set out to investigate the treatment effect on neutrophils and eosinophils and the relationship of anti-inflammatory effects to clinical outcomes (lung function as assessed by forced expiratory volume in 1 s (FEV1) and health-related quality of life as assessed by the Chronic Respiratory Questionnaire (CRQ)) in patients with COPD.

METHODS

Patient population
Sixty subjects with a clinical diagnosis of COPD were recruited between September 2002 and April 2005 from two respiratory centres, the Montreal Chest Institute and Hôpital Laval, Canada. Subjects were eligible for this study if they met the following criteria: age ≥40 and ≤75 years; smoking history (≥10 pack-years); post-bronchodilator FEV1 ≥25% of predicted value and FEV1/forced vital capacity (FVC) <0.70; no history of asthma, atopy (as assessed by an allergy skin prick test during screening) or any other active lung disease. Patients on home oxygen or with raised carbon dioxide tension (>44 mm Hg),...
of the CRQ were performed at visit 2 and after 4 and 12 weeks of treatment. The ATS-DLD questionnaire was administered possibly related to the study treatment).

code, to ensure that the treatment code would be broken only established by GEREQ, who were in possession of the treatment numbers which was stratified by centre and which used a block performed using a central computer generated list of random matches placebo Diskus twice daily. Randomisation was m

CD8

BMK13) and neutrophils (elastase; DAKO NP57) were used. In order to phenotype the inflammatory cells present in the endobronchial biopsy specimens were obtained according to American Thoracic Society guidelines. Six specimens from the endobronchial biopsy specimens were obtained according to the number of neutrophils and eosinophils in bronchial lavage (BAL) fluid was collected following the bronchoscopy procedure and sputum induction was performed on three occasions (2–4 days before bronchoscopy at randomisation, after 4 weeks of treatment and at the end of treatment); analysis of these samples has not yet been completed and will be the subject of a future publication.

Tissue preparation

With the use of an Olympus BF fibreoptic bronchoscope, endobronchial biopsy specimens were obtained according to American Thoracic Society guidelines. Six specimens from the subsegmental bronchus were obtained from the right lower, middle and upper lung using cup forceps. In preparation for immunocytochemistry, the bronchial biopsies were fixed accordingly and processed as either frozen sections or paraffin embedded sections as previously described. Briefly, frozen sections were thawed, blocked in Universal Blocking Solution (DAKO Diagnostics) for 15 min and incubated with optimum concentration of the primary antibody overnight at 4°C in a humidified chamber. The next day the slides were washed in Tris-buffered saline solution (pH 7.2), incubated with secondary antibody, and finally incubated with mouse alkaline phosphatase antialkaline phosphatase (DAKO). The reactions were then visualised by adding alkaline phosphatase substrate to the Fast Red (Sigma Chemical Company). Paraffin embedded tissue sections were stained using the strepavidin biotin peroxidase procedure. Briefly, tissue sections were deparaffinised, dehydrated and blocked in 3% hydrogen peroxide for 5 min. Antigen retrieval was enhanced by placing sections in a heat-induced epitope retrieval solution (EDTA buffer pH 8.0 for CD8 detection; Tris buffer pH 10 for CD68). Primary antibody was then applied for 1 h at room temperature. A goat anti-rabbit secondary antibody conjugated to hors eradish peroxidase (DAKO K377) was then applied. A coloured reaction was achieved by treating the slides with 3,3 diaminobenzidine (Sigma D5637). For negative controls the primary antibody was replaced by an isotype-matched control antibody.

Not all samples were stained at once but were done in batches; however, we made sure that pre and post biopsies from each individual subject were stained in the same run. Similarly, cell counting was delayed until there were sufficient pre and post biopsies to be randomised. The number of positive cells was averaged from six to eight random non-overlapping fields from two biopsy samples per subject and expressed as the mean number of positive cells per mm². Cell counts were performed blind by two independent observers using an Olympus light microscope (Carson Group, Markham, Ontario, Canada) with <400× magnification with an eyepiece graticule of 0.202 mm². Observers were blinded not only to drug treatment but also to whether the biopsies were pre or post treatment. The level of agreement between blinded counters was >90%. The results of cell counts from the two counters were averaged.

Statistical methods

All data management and data analyses were performed by the authors. The main outcome variables in this study were the treatment difference in the numbers of CD8+ T lymphocytes and CD68+ macrophages in bronchial biopsies. Each end point has been analysed as a change from baseline. The primary treatment comparisons were between SFC and placebo, and FP and placebo, and secondary treatment comparisons were between SFC and FP. The secondary outcome variables were the number of neutrophils and eosinophils in bronchial biopsies. Since, at the time of starting this trial, no previous study using a combination of inhaled corticosteroid and long-acting β2 agonist had assessed the airway inflammatory response in COPD, the sample size was difficult to quantify. Based on a previous study and similar studies in asthma, we estimated the number of patients to be 20 per treatment group for the primary comparisons. Confidence intervals were provided to estimate the size of any treatment effects and these were used to determine the clinical relevance of the results. Statistical analysis was performed using data processed by R, a language and environment for statistical computing (R Foundation for Statistical Computing; Vienna, Austria). All available on-treatment data were included in the summary and analyses. As expected, some of the data were not normally distributed, but the difference between the results post-treatment minus pretreatment passed the test for normality in all cases. As such, data were analysed by Anova tests with Tukey honest significant comparisons a posteriori. Since the

Effect of salmeterol/fluticasone propionate on airway inflammation in COPD
main outcome was limited to CD8+ T lymphocytes and CD68+ macrophages in bronchial biopsies and the different end points in this study were considered to represent separate pathways, they were treated independently and no adjustments for multiplicity were performed.

RESULTS
Sixty eligible subjects were randomised to treatment, 19 to the SFC treatment arm, 20 to the FP arm and 21 to the placebo arm (fig 1). Five subjects refused to have bronchoscopy performed and four subjects did not provide a post-treatment biopsy

<table>
<thead>
<tr>
<th>Table 1 Baseline characteristics of study patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SFC</strong> (n = 19)</td>
</tr>
<tr>
<td>Mean (SD) age (years)</td>
</tr>
<tr>
<td>Male/female (n)</td>
</tr>
<tr>
<td>Current/former smokers (n)</td>
</tr>
<tr>
<td>Mean (SD) pack-years</td>
</tr>
<tr>
<td>Mean (SD) post-BD FEV1 (% predicted)</td>
</tr>
<tr>
<td>Mean (SD) BD reversibility (%)</td>
</tr>
<tr>
<td>Mean (SD) TLCO (%)</td>
</tr>
<tr>
<td>GOLD stages (n)</td>
</tr>
<tr>
<td>I: FEV1 &gt;80%</td>
</tr>
<tr>
<td>II: 50% &lt; FEV1 &lt;80%</td>
</tr>
<tr>
<td>III-IV: FEV1 &lt;50%</td>
</tr>
<tr>
<td>Respiratory medications before study (n)</td>
</tr>
<tr>
<td>Short-acting bronchodilators</td>
</tr>
<tr>
<td>Long-acting anticholinergic agent</td>
</tr>
<tr>
<td>Long-acting β2 agonists</td>
</tr>
<tr>
<td>Inhaled corticosteroids</td>
</tr>
<tr>
<td>Long-acting β2 agonists/inhaled corticosteroids</td>
</tr>
<tr>
<td>Theophylline</td>
</tr>
</tbody>
</table>

*SFC, salmeterol xinafoate/fluticasone propionate; FP, fluticasone propionate; BD, bronchodilator; FEV1, forced expiratory volume in 1 s; TLCO, carbon monoxide transfer factor.

*FEV1 reversibility calculated as: post-BD FEV1 (l) – pre-BD FEV1 (l)/pre-BD FEV1 (l) x 100%.
because they withdrew prematurely. Biopsy data before and after treatment were therefore available for analysis from 51 subjects (19 treated with SFC, 17 treated with FP and 15 treated with placebo). One patient in the placebo group received treatment for only 2 months.

The demographic characteristics, smoking history, baseline lung function, respiratory symptoms and prior respiratory medications of the randomised patients are summarised in table 1. Patients were primarily male, half of the patients were current smokers, and most of them (83%) had a disease severity of GOLD (Global initiative for Obstructive Lung Disease) stages II–IV.21

Primary end point
Following 12 weeks of treatment, a statistically significant fall from baseline in the numbers of CD8+ T lymphocytes (p<0.001) and CD68+ macrophages (p = 0.008) was seen after treatment with SFC but not with FP alone (table 2). As shown in fig 2, the mean differences in the number of CD8+ T lymphocytes and CD68+ macrophages between SFC and placebo were statistically significant (p<0.001 and p = 0.03, respectively). The mean differences in CD8+ T lymphocytes and CD68+ macrophages between FP and placebo failed to reach statistical significance. The mean differences in CD8+ T lymphocytes and CD68+ macrophages between SFC and FP were statistically significant (p = 0.01 and p = 0.04 for CD8+ cells and CD68+ cells, respectively).

Secondary end points
Neither treatment had significant effects on the low numbers of biopsy neutrophils and eosinophils, although there was an increase from baseline in neutrophil numbers with FP that approached statistical significance (p = 0.07, table 2). Although there was no significant difference in the number of neutrophils between SFC and placebo, the number of neutrophils was significantly lower for SFC than for FP alone (p<0.001, fig 2). Furthermore, the comparison between FP and placebo showed a lower number of neutrophils in the placebo arm (p = 0.005). No statistically significant differences were seen between treatments for the number of eosinophils.

Clinical outcomes
No evidence of improvement in clinical outcomes was observed as measured by lung function as well as health-related quality of life questionnaires. The post bronchodilator % predicted FEV1 was similar at baseline and after treatment in the SFC and FP treatment group and a decrease of 6% from baseline was seen in the placebo group. Clinically, all treatments were well tolerated and only one patient (treated with FP) experienced a drug-related adverse event (candidiasis). Three patients experienced post-bronchoscopy pneumonitis that was thought to be associated with the BAL procedure.

DISCUSSION
Our study has shown that the combination of salmeterol and FP reduces aspects of airway inflammation in patients with COPD and that these effects are not observed with FP alone. We have demonstrated a reduction in CD8+ T lymphocytes and CD68+ macrophages following 12 weeks of treatment with combination therapy. Both these cell types appear to be important in the pathogenesis of COPD.26 Treatment with FP did not have these effects, giving only small non-significant decreases in the number of CD8+ cells compared with placebo and having no effects on CD68+ macrophages. Combination treatment reduced neutrophil number counts compared with FP treatment but not compared with placebo. We did not demonstrate an effect of either active treatment on eosinophils in bronchial biopsies. The numbers of eosinophils observed in the airways of these patients with stable COPD was low and our data do not provide support for an important role for these cells in stable COPD.

COPD is recognised as an inflammatory disease21 so treatments that reduce inflammatory cells in patients with COPD may be of importance in therapeutic management. CD8+ cells in the airways and lung parenchyma have been shown to correlate significantly with the degree of airflow limitation, which suggests that CD8+ cells may be related to the progression of the disease.4

Cytotoxic CD8+ cells have the potential to release mediators such as tumour necrosis factor α, interferon γ, perforins and granzymes, leading to lung destruction. Increased macrophage...
numbers in the airways are associated with parenchymal destruction and COPD severity. CD68 macrophages mainly release tumour necrosis factor α, a proinflammatory cytokine which is involved in systemic inflammation as well as tissue hypoxia and muscle wasting seen in COPD.

Reduction of CD8+ cells by SFC in the airways of patients with COPD has also been demonstrated in a recent study by Barnes and colleagues. That study, however, did not include an inhaled corticosteroid treatment group so our results, in addition to confirming the effects of combination treatment on CD8+ cells, also provide evidence that, in large part, the effect is not related to the action of inhaled corticosteroids alone. In contrast to these investigators, we also observed an effect of combination treatment on CD8+ macrophages, providing further evidence for enhanced anti-inflammatory effects of combination treatment. Effects on macrophages have not been observed with inhaled corticosteroids alone. Our results demonstrating an increased number of neutrophils following FP treatment were not surprising, yet they further support the possibility that corticosteroids inhibit neutrophilic apoptosis, thus prolonging their survival, as previously shown. The failure to show a significant change in neutrophil counts following combination treatment, however, may have resulted from the ability of salmeterol to reduce the number of neutrophils by promoting their apoptosis and reducing levels of interleukin-8, a key neutrophil chemoattractant. Although these effects have been well established in vitro and in asthma, only a salmeterol treatment arm in this study design could confirm this possibility in COPD.

One limitation of the study is that we cannot extrapolate that the inflammatory changes are responsible for the improvement in clinical outcomes shown in previous studies. For example, in the study by Barnes et al., the anti-inflammatory effect of SFC was accompanied by significant improvements in lung function. Our study was not powered to detect an effect on clinical outcomes and, furthermore, the study was of relatively short duration which may have limited the possibility of showing any benefit on quality of life. This study was also not designed to assess the effects on COPD exacerbations; previous studies have shown that a reduction in exacerbations has been observed with combination therapy. The biopsy specimens in this study were taken from the proximal rather than the distal airways, which is a clear limitation as the proximal airways may not closely reflect all the pathological changes present in the peripheral airways and lung parenchyma, which are the sites generally responsible for airflow limitation in COPD. However, increases in CD8+ cells have been observed in both the proximal and distal airways where a relationship between their numbers and reduced lung function has been shown. As such, the site of the biopsy is possibly the reason for the discrepancy in inflammatory and clinical responses.

Subjects were allowed to use short-acting β2 agonists both during the run-in period and during the study. Although one of the measures of interest was the interaction between inhaled corticosteroids and β2 agonists, this is unlikely to have biased the results. Randomisation will tend to make study groups comparable and patients were kept blind to the assigned treatment. There is increasing evidence to suggest a synergistic and/or additive effect of long-acting β2 agonists and inhaled corticosteroids on airway inflammation. Although the mechanisms for this observation are not entirely clear, in vitro studies suggest that corticosteroids may regulate β2 receptor function by increasing expression of the receptor and by restoring G protein/β2 receptor coupling and inhibiting β2 receptor downregulation. By modulating glucocorticoid receptor phosphorylation, long-acting β2 agonists prime the glucocorticoid receptor and affect its nuclear localisation. This interactive mechanism presumably leads to additive and/or synergistic effects of combination therapy on airway inflammation. Long-acting β2 agonists may also have non-bronchodilator effects which are anti-inflammatory. They have been shown to inhibit mediator release from macrophages and, in patients with asthma, to reduce the number of neutrophils in bronchial biopsies. The design of our study does not allow us

![Table 2: Biopsy cell counts (cells/mm²) according to treatment allocation](http://thorax.bmj.com/)

<table>
<thead>
<tr>
<th></th>
<th>SFC (n = 19)</th>
<th>FP (n = 17)</th>
<th>Placebo (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD8+ T lymphocytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline mean</td>
<td>191 (79)</td>
<td>195 (69)</td>
<td>187 (43)</td>
</tr>
<tr>
<td>Post-treatment mean</td>
<td>107 (31)</td>
<td>164 (47)</td>
<td>201 (48)</td>
</tr>
<tr>
<td>Mean change from baseline</td>
<td>-84 (67)</td>
<td>-31 (47)</td>
<td>14 (41)</td>
</tr>
<tr>
<td>Median change from baseline</td>
<td>-75 (-110 to -34)</td>
<td>-24 (-51 to -15)</td>
<td>11 (-4 to 41.50)</td>
</tr>
<tr>
<td>p Value for mean change from baseline</td>
<td>&lt;0.001</td>
<td>0.20</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>CD68+ macrophages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline mean</td>
<td>102 (37)</td>
<td>122 (49)</td>
<td>135 (53)</td>
</tr>
<tr>
<td>Post-treatment mean</td>
<td>74 (24)</td>
<td>122 (49)</td>
<td>136 (40)</td>
</tr>
<tr>
<td>Mean change from baseline</td>
<td>-29 (22)</td>
<td>0.33 (33)</td>
<td>2 (49)</td>
</tr>
<tr>
<td>Median change from baseline</td>
<td>-30 (-41 to -10)</td>
<td>-2 (-15 to 30)</td>
<td>20 (-38 to 34)</td>
</tr>
<tr>
<td>p Value for mean change from baseline</td>
<td>0.008</td>
<td>0.98</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>Neutrophils</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline mean</td>
<td>50 (19)</td>
<td>53 (20)</td>
<td>41 (26)</td>
</tr>
<tr>
<td>Post-treatment mean</td>
<td>41 (19)</td>
<td>67 (24)</td>
<td>36 (21)</td>
</tr>
<tr>
<td>Mean change from baseline</td>
<td>-9 (18)</td>
<td>14 (12)</td>
<td>-4 (15)</td>
</tr>
<tr>
<td>Median change from baseline</td>
<td>-11 (-19 to 0.5)</td>
<td>18 (6 to 21)</td>
<td>-4 (-11 to 7.5)</td>
</tr>
<tr>
<td>p Value for mean change from baseline</td>
<td>0.16</td>
<td>0.07</td>
<td>0.62</td>
</tr>
<tr>
<td><strong>Eosinophils</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline mean</td>
<td>5 (5)</td>
<td>9 (7)</td>
<td>7 (4)</td>
</tr>
<tr>
<td>Post-treatment mean</td>
<td>5 (4)</td>
<td>8 (7)</td>
<td>9 (5)</td>
</tr>
<tr>
<td>Mean change from baseline</td>
<td>-0.4 (5)</td>
<td>-1 (6)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Median change from baseline</td>
<td>0 (-3 to 3)</td>
<td>-1 (-2 to 3)</td>
<td>1 (0.5 to 7)</td>
</tr>
<tr>
<td>p Value for mean change from baseline</td>
<td>0.80</td>
<td>0.61</td>
<td>0.09</td>
</tr>
</tbody>
</table>

SFC, salmeterol xinafoate/fluticasone propionate; FP, fluticasone propionate. Data expressed as mean (SD) or median (interquartile ranges).
to define the effects of treatment with salmeterol alone in patients with COPD.

In summary, this study provides evidence of anti-inflammatory effects in patients with COPD for the combination of an inhaled corticosteroid with a long-acting $\beta_2$ agonist that are not observed with inhaled corticosteroid alone. The enhanced effects of combination therapy on key inflammatory cells may explain the clinical efficacy that has been shown in previous trials of COPD. Combination therapy reduced CD8+ cytotoxic T lymphocytes and CD68+ macrophages, cells that have been shown to be correlated with COPD disease severity and recognised to be important in the pathogenesis of COPD. However, a long-term study will be needed to determine whether combination therapy can modify the progressive nature of this disorder.

ACKNOWLEDGEMENTS

The authors acknowledge the support of Martine Bélanger RN, Hôpital Laval, Institut Universitaire de Cardiologie et de Pneumologie de l'Université Laval; and Leo Cicora RT and Elizabeth Sukhdeo RT, Montreal Chest Institute, McGill University Health Centre.

Authors' affiliations

Jean Bourbeau, Pato Christodouloupolous, Ronald Olivenstein, Respiratory Epidemiology and Clinical Research Unit, Montreal Chest Institute, McGill University Health Centre, Montréal, Québec, Canada

François Maltais, Centre de recherche, Hôpital Laval, Institut Universitaire de cardiologie et de pneumologie, Université Laval, Québec, Canada

Yasuhiro Yamauchi, Qutayba Hamid, University Health Centre, Montréal, Quebec, Canada

This study was funded by an unrestricted research grant from GlaxoSmithKline.

Competing interests: None.

REFERENCES


40 Johnson JM, Rennard S. Alternative mechanisms for long-acting $\beta_2$-agonist resistant COPD. Chest 2001;120:588–70.

Effect of salmeterol/fluticasone propionate on airway inflammation in COPD: a randomised controlled trial

Jean Bourbeau, Pota Christodoulopoulos, Francois Maltais, Yasuhiro Yamauchi, Ronald Olivenstein and Qutayba Hamid

Thorax 2007 62: 938-943 originally published online June 8, 2007
doi: 10.1136/thx.2006.071068

Updated information and services can be found at:
http://thorax.bmj.com/content/62/11/938

These include:

References
This article cites 40 articles, 6 of which you can access for free at:
http://thorax.bmj.com/content/62/11/938#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Inflammation (1020)
- Cardiothoracic surgery (676)
- Clinical trials (epidemiology) (557)
- Drugs: respiratory system (526)

Notes