

## Flurbiprofen does not affect elastase-induced bronchial secretory cell metaplasia in hamsters

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*Flurbiprofen does not affect elastase-induced bronchial secretory cell metaplasia in hamsters. H.M. Nields, G.L. Snider, T.G. Christensen.*

**ABSTRACT:** The anti-inflammatory drug flurbiprofen speeds the repair of cigarette smoke-induced bronchial secretory cell metaplasia (SCM) in rats. We tested whether flurbiprofen would prevent or mitigate the development of elastase-induced bronchial SCM in hamsters. Twice daily injections of flurbiprofen (4 or 6 mg·kg<sup>-1</sup>·day<sup>-1</sup>) were begun immediately after intratracheal instillation of porcine pancreatic elastase (PPE) or its vehicle, saline. At 3 wks, the bronchi of these animals were compared to those of animals receiving PPE or saline. PPE-treated hamsters, with or without flurbiprofen, developed moderate to severe SCM. Control hamsters had normal airways. Flurbiprofen had no effect on the neutrophilic pulmonary infiltrate seen 2 h after PPE instillation. We conclude that the development of elastase-induced SCM in hamsters is not affected by a flurbiprofen regimen begun directly after elastase instillation. The pathogenesis of this lesion may involve factors which are insensitive to flurbiprofen or which trigger the lesion immediately upon exposure to the enzyme.

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Secretory cell metaplasia (SCM) is induced in the intrapulmonary bronchi of hamsters by a single intratracheal instillation of porcine pancreatic elastase (PPE) or human neutrophil elastase (HNE) [1, 2]. A similar histological change occurs in rats exposed to chronic cigarette smoke [3] or sulphur dioxide [4, 5]. Elastase-induced change in hamster bronchi is recognized by the accumulation of secretory granules within nonciliated epithelial cells [6-8]. Our ultrastructural observations showing dilated rough endoplasmic reticulum and active Golgi apparatus suggest that granule accumulation results from up-regulation of granule synthesis by elastase [6, 9].

Flurbiprofen (2-(2-fluoro-4-biphenyl) propionic acid), a non-steroidal anti-inflammatory agent, was shown to hasten the recovery of cigarette smoke-induced SCM in rat intrapulmonary airways; treatment began after cessation of smoke exposure [10]. Because of flurbiprofen's effectiveness in down-regulating granule accumulation by rat bronchial epithelial cells, we tested whether it could prevent or mitigate up-regulation of granule accumulation by hamster bronchial secretory cells exposed to PPE. We found that twice daily injections of flurbiprofen begun immediately after PPE instillation had no effect on this bronchial lesion in hamsters.

### Methods

Male Syrian Golden hamsters, weighing 130-150 g, were purchased from Charles River Laboratories and were fed *ad libitum* with Purina Rat Chow. Porcine pancreatic elastase (Sigma, Type IV, lot No. 96F-8005) was dissolved in unbuffered physiological saline. The enzyme was prepared on the day of use.

Flurbiprofen was a gift from the Upjohn Co. (U-27182, lot No.5290A). It was dissolved in 1% NaHCO<sub>3</sub> and sterilized by filtration through 0.2 µm pore filters. A fresh drug solution was made at the beginning of each treatment week. Drug solutions were of two concentrations, 1.5 mg·ml<sup>-1</sup> (4 mg·kg<sup>-1</sup>·day<sup>-1</sup>) and 2.25 mg·ml<sup>-1</sup> (6 mg·kg<sup>-1</sup>·day<sup>-1</sup>), to ensure that each drug treatment group received the same injection volumes. Solutions were divided into daily aliquots and stored at 4° C. Each daily aliquot was brought to room temperature before injection. Intraperitoneal injections of drug or 1% NaHCO<sub>3</sub> were begun immediately after enzyme or saline instillation and were continued twice daily for 21 days.

Thirty hamsters were divided into 5 treatment groups. Eighteen hamsters received single intratracheal instillation of 360 µg PPE in 0.5 ml saline.

Six of these animals were treated with flurbiprofen  $4 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ , 6 were treated with flurbiprofen  $6 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  and 6 were treated with 1%  $\text{NaHCO}_3$  alone. The remaining 12 hamsters were instilled intratracheally with saline. Six of these animals were treated with flurbiprofen  $6 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  and 6 were treated with 1%  $\text{NaHCO}_3$  alone. Animals were weighed weekly and drug dose adjusted accordingly.

Three weeks after instillation all hamsters were killed by exsanguination after Rompun/Ketamine-induced anaesthesia (0.2 ml per hamster) and a local ventral surface line block with Lidocaine. Lungs were inflation-fixed by injecting 5 ml of 4% formalin/1% glutaraldehyde in 0.1 M Na cacodylate buffer pH 7.3 slowly into the trachea. Lungs and tracheas were removed and fixed en bloc overnight. Three 4 mm thick transverse slices were cut from the hilar region of the left lung of each animal. Tissue was degassed, dehydrated and embedded in paraffin. Three 4  $\mu\text{m}$  bronchial cross-sections were cut from each block and stained with alcian blue pH 2.0/periodic-acid-Schiff. Secretory cell index (SCI) values were determined by microscopic analyses performed separately by three observers without knowledge of the treatment groups. The observers' values for each animal were averaged. Secretory cell indices (SCIs) were determined based on a series of standard slides graded 0–4 in order of the increasing amount and intensity of their intracellular blue or magenta reaction product [1]. Grade 0 represents no SCM, grade 1 mild, grade 2 moderate, grade 3 severe and grade 4 very severe. Data were initially analysed using the non-parametric Kruskal-Wallis test [11]. Significant differences between groups were identified by pairwise comparisons using the Mann-Whitney rank-sum test with Bonferroni adjustment [12]. Differences between treatment groups were considered significant at  $p < 0.05$ .

An additional experiment was performed to determine whether administration of flurbiprofen immediately following PPE instillation would reduce the early pulmonary leucocytic infiltrate known to occur in this model [13]. Nine hamsters were divided into 3 treatment groups. Six hamsters each received a single 0.5 ml (360  $\mu\text{g}$ ) PPE instillation. Immediately after instillation, 3 of these animals were given intraperitoneal injections of flurbiprofen ( $3 \text{ mg}\cdot\text{kg}^{-1}$ ) and 3 were treated with  $\text{NaHCO}_3$  alone. The remaining 3 hamsters received a single 0.5 ml intratracheal instillation of saline and were treated once intraperitoneally with  $\text{NaHCO}_3$ .

All animals were killed 2 h later by exsanguination after anaesthesia. A small bore polyethylene tube, attached to an 18 gauge needle was inserted into the trachea of each animal and was tied in place. Three aliquots of 5 ml of physiological saline were instilled into the lungs and withdrawn slowly. The fluid collected was kept on ice. The lungs were then removed and were inflation-fixed with 4% formalin/1% glutaraldehyde in 0.1 M Na cacodylate buffer. Transverse sections were cut from the hilar region of

the left lungs. Tissue was dehydrated through graded alcohols and embedded in glycol methacrylate. Sections 3  $\mu\text{m}$  in thickness were stained with tissue Giemsa.

The bronchoalveolar lavage fluid (BAL) was centrifuged at  $300 \times g$  for 10 min. Cell pellets were resuspended in 2 ml of physiological saline. Fifty microlitres of this suspension was added to 100  $\mu\text{l}$  of 0.10 M HCl to lyse erythrocytes; the leucocytes were counted in a haemocytometer and the total number of leucocytes lavaged was calculated. Slides prepared from the cell suspension in a cytospin centrifuge (Shandon) were stained with Wright's stain and the percentage of neutrophils determined in 200 cells. Data were analysed using analysis of variance [11]. Differences between treatment groups were considered significant at  $p < 0.05$ .

## Results

The SCI values for large intrapulmonary airways are shown in figure 1. There was a significant increase in SCI values for all PPE-treated groups over saline-treated groups, ( $p < 0.05$ ). There was no effect of flurbiprofen, at either dose, on PPE-induced SCM.

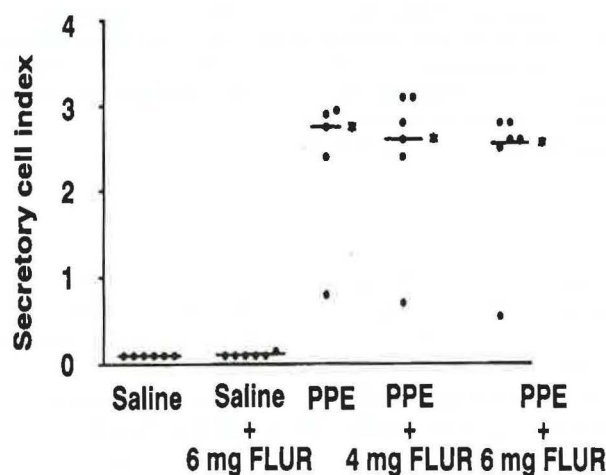


Fig. 1. — Secretory cell indices (SCIs) determined from hamster mainstem intrapulmonary bronchi 21 days after a single intratracheal instillation of saline (0.5 ml) or PPE (360  $\mu\text{g}\cdot 0.5 \text{ ml}^{-1}$  saline) and daily intraperitoneal injections of flurbiprofen (FLUR) at  $4 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  or  $6 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  or 1%  $\text{NaHCO}_3$ . SCIs were determined by grading sections semi-quantitatively on a scale of 0–4 according to the increasing amount and intensity of AB/PAS intracellular staining of airway surface epithelium. One hamster treated with only PPE died within 12 h after PPE instillation. On microscopic examination the hamster showed massive pulmonary haemorrhage. The median value for each group is indicated by the horizontal line. \*: significantly different from saline control group at  $p < 0.05$ ; PPE: porcine pancreatic elastase; AB: alcian blue; PAS: periodic-acid-Schiff.

Data acquired from analysis of BAL fluid in the animals killed at 2 h are shown in figure 2. PPE-treated animals with or without flurbiprofen treatment showed significantly increased numbers of leucocytes when compared with saline-treated controls, ( $p < 0.05$ ). Flurbiprofen produced no significant change in the

number of lavageable leucocytes from PPE-treated hamsters. There was no significant difference between the average percentage of neutrophils counted in the lavage fluid from hamsters treated with PPE + flurbiprofen (40.8%) and that from the hamsters treated with PPE +  $\text{NaHCO}_3$  (39.8%). The cytopsin preparation from saline-treated hamsters had insufficient cell numbers to allow a differential cell count.

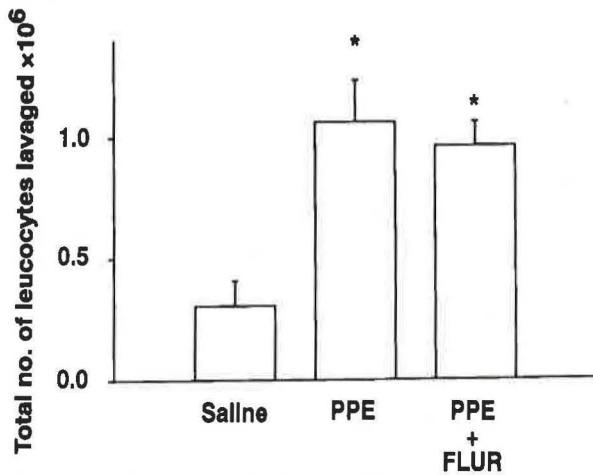


Fig. 2. - Bronchoalveolar lavage results are shown from hamsters 2 h after intratracheal instillation of saline (0.5 ml) or PPE (360  $\mu\text{g}\cdot 0.5 \text{ ml}^{-1}$  saline) with or without immediate intraperitoneal injection of flurbiprofen (FLUR). Individual values are expressed as the mean of 3 animals  $\pm$ SE. \*: significantly different from saline control group at  $p < 0.05$ ; PPE: porcine pancreatic elastase.

Histological examination of hamster lungs, 2 h after PPE instillation revealed severe haemorrhage within conducting airways and alveoli, and surrounding blood vessels. A neutrophil influx was present within the alveoli and many neutrophils were seen attached to and penetrating the walls of the pulmonary veins. In contrast, only a few neutrophils were seen in the mucosa of the mainstem bronchus. No differences were apparent between flurbiprofen and  $\text{NaHCO}_3$ -treated hamsters.

### Discussion

These results demonstrate that flurbiprofen given at doses up to  $6 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  for 21 days after PPE instillation does not affect the development of PPE-induced SCM in the large intrapulmonary bronchi of hamsters. The dose used in this experiment was 1.5 times the dose used to speed recovery of cigarette smoke-induced SCM in rats [10] and 5 times, 12 times, 6 times, and 4.5 times greater than the doses which produced various anti-inflammatory effects in rats, guinea-pigs, mice and humans, respectively, [14, 15]. It seems unlikely that the failure of flurbiprofen to affect SCM in our hamsters resulted from an insufficient dose of drug.

Because flurbiprofen was first injected immediately after PPE instillation it is possible that early events

relevant to SCM occurred before flurbiprofen reached protective levels in the hamster airways. Peak plasma levels of flurbiprofen occur in man 1.5 h after oral dosing and anti-pyretic activity in rats is produced by 2 h after oral dosing [14, 15]. Intraperitoneal injection should have produced peak levels of the drug at an earlier time. However, BAL results in this study showed that flurbiprofen injection had no measurable effect on the neutrophil infiltration in hamster lungs 2 h after PPE instillation.

While the timing of flurbiprofen treatment may account for the absence of an early anti-inflammatory effect, it is unclear why the drug did not mitigate the full development of PPE-induced SCM, which requires 16–24 days [6]. Within 4 days after initiating flurbiprofen treatment, the elevated number of secretory cells seen in rat bronchi exposed to cigarette smoke had returned to normal [10]. It would appear, therefore, that 21 days of flurbiprofen treatment allowed ample time for the drug to exert an ameliorative effect on granule accumulation in hamster bronchi. Alternatively, elastase could act instantly by triggering a chain of metabolic events leading to accumulation of granules. This up-regulation of granule synthesis could be insensitive to flurbiprofen regardless of time or length of delivery.

In contrast to its ineffectiveness in the hamster SCM model, flurbiprofen appears to accelerate the down-regulation of rat secretory granule synthesis upon cessation of cigarette exposure [10]. The mechanism of this action has not been elucidated. Flurbiprofen inhibits cyclo-oxygenase and random migration of neutrophils *in vitro* and decreases prostaglandin levels and neutrophil influx *in vivo* [15–20]. It is uncertain, however, whether neutrophils and their enzymes play an important role in inducing or maintaining cigarette smoke-induced SCM in rats.

The failure of flurbiprofen to affect PPE-induced SCM in the hamster suggests that mechanisms regulating granule accumulation by bronchial epithelial cells are different in the rat and hamster models of SCM. Cigarette smoke may cause SCM in rats by an indirect mechanism which may be mediated by chemical substances such as prostaglandins. In contrast, SCM is induced in the hamster bronchus by a single instillation of elastase. Studies in this laboratory have demonstrated ultrastructural changes in bronchial cell surfaces [9] and changes in surface lectin binding at 2 h after human neutrophil elastase instillation [21]. These data suggest that elastase may induce SCM as a result of structural alterations in the surface of the hamster bronchial epithelium. Whether similar cell surface changes occur in rat bronchi exposed to cigarette smoke is unknown.

The difference between flurbiprofen effects on SCM induced by cigarette smoke in the rat and by PPE in the hamster could be due to differences in the composition of the epithelial cells in the two species or to differences in the injury induced by the agents. SCM is induced in the rat by chronic exposure to cigarette smoke and does not appear to involve acute lung

injury [3, 22–25]. The airway lesion can be prevented by treatment with a number of steroidal and non-steroidal, anti-inflammatory agents [10, 26, 27].

It is of interest that dexamethasone inhibits induction of SCM by human neutrophil products in the rat trachea [28]. The effect of corticosteroids on SCM caused by elastase in hamster bronchi is unknown. Additional experimental studies are needed to reveal the mechanisms involved in the pathogenesis of elastase-induced SCM.

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*Le flurbiprofen n'influence pas la métaplasie des cellules sécrétoires bronchiques induite par l'élastase chez les hamsters. H.M. Nields, G.L. Snider, T.G. Christensen.*

RÉSUMÉ: Le produit anti-inflammatoire flurbiprofen accélère la réparation de la métaplasie des cellules sécrétoires bronchiques induite par la fumée de cigarette chez les rats. Nous avons étudié si le flurbiprofen pouvait prévenir ou limiter le développement de cette métaplasie induite

par l'élastase chez les hamsters. Des injections biquotidiennes de flurbiprofen (4 ou 6 mg·kg<sup>-1</sup>·jour<sup>-1</sup>) ont été commencées immédiatement après l'instillation intratrachéale d'élastase pancréatique porcine (PPE) ou de son véhicule, la solution saline isotonique. A la troisième semaine, les bronches de ces animaux ont été comparées à celles d'animaux ayant reçu PPE ou solution saline. Les hamsters traités au PPE, avec ou sans flurbiprofen, ont développé une métaplasie sécrétoire modérée à sévère. Les hamsters de contrôle avaient des voies aériennes normales.

Le flurbiprofen n'a pas d'effet sur l'infiltrat pulmonaire à neutrophiles observé 2 h après l'instillation de PPE. Nous concluons que le développement d'une métaplasie sécrétoire induite par l'élastase chez les hamsters, n'est pas influencé par un régime à base de flurbiprofen commencé immédiatement après l'instillation d'élastase. La pathogénie de cette lésion pourrait impliquer des facteurs qui ne sont pas sensibles au flurbiprofen ou qui diluent la lésion immédiatement après l'exposition à l'enzyme.  
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