Effect of Ibuprofen on Neutrophil Migration in Vivo in Cystic Fibrosis and Healthy Subjects

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ABSTRACT

Long-term treatment with ibuprofen twice daily, at doses that achieve peak plasma concentration (C_{max}) > 50 \mu g/ml, slows progression of lung disease in patients with cystic fibrosis (CF). Previous data suggest that C_{max} > 50 \mu g/ml is associated with a reduction in neutrophil (PMN) migration into the lung and that lower concentrations are associated with an increase in PMN migration. To estimate the threshold concentration at which ibuprofen is associated with a decrease in PMN migration in vivo, we measured the PMN content of oral mucosal washes in 35 healthy (age 19–40 years) and 16 CF (age 18–32 years) subjects who took ibuprofen twice daily for 10 days in doses of 2 to 3, 5 to 10, 13 to 17, and 20 to 30 mg/kg, administered orally, twice daily for 10 days, compared with a 3-day baseline period. We also assessed the duration of effect and possible rebound.

The inflammatory response to chronic endobronchial infection in patients with cystic fibrosis (CF) is characterized by persistent neutrophil (PMN) influx, which contributes to lung destruction (Konstan and Berger, 1993; Konstan and Berger, 1997). Ibuprofen, in high doses (those producing peak plasma concentrations > 50 \mu g/ml), inhibits the migration, adherence, swelling, and aggregation of PMNs, as well as the release of lysosomal enzymes (Brown and Collins, 1977; Spisani et al., 1978; Higgs et al., 1980; Flynn et al., 1984; Kaplan et al., 1984; Maderazo et al., 1984; Shimanuki et al., 1985; Venezio et al., 1985). High doses of ibuprofen have also been associated with decreased PMN influx in several animal models of pulmonary infection and inflammation (Sordelli et al., 1985; Rinaldo and Pennock, 1986). Thus, ibuprofen has been proposed as a strategy to preserve lung function in CF (Konstan, 1996). In a 4-year randomized, double-blind, placebo-controlled trial of high-dose ibuprofen in patients with CF, ibuprofen, taken orally twice daily, in doses sufficient to achieve peak plasma concentrations of 50 to 100 \mu g/ml, significantly slowed the progression of lung disease (Konstan et al., 1995).

Our hypothesis is that the protective effect of ibuprofen is due to decreasing PMN migration into the lung. In this study, we used PMN migration to the oral mucosa as a surrogate marker for PMN migration to the lower airway mucosa to explore the relationship between ibuprofen and its effect on PMN migration. This site has been used in previous studies to demonstrate the impact of corticosteroids on PMN migration to an inflammatory site (Wright et al., 1986). The present study addressed three questions relative to the optimal use of ibuprofen for CF: How precisely does the dose need to be adjusted? Is twice daily dosing sufficient to decrease PMN migration? After the drug is discontinued, does PMN migration increase above baseline (rebound)?

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ABBREVIATIONS: CF, cystic fibrosis; PMN, neutrophil; NSAID, nonsteroidal anti-inflammatory drug; AUC, area under the plasma concentration-time curve.
rebound effect) of ibuprofen on PMN migration to the oral mucosa in CF patients and healthy controls. The response was measured for at least 3 days after discontinuing ibuprofen and compared with the treatment period.

Although this study was designed with the intent of applying the results for use of ibuprofen for the treatment of CF lung disease, the information gained from this study could prove useful for a number of other conditions in which ibuprofen is used to treat inflammation.

Materials and Methods

Subjects. Subjects consisted of 35 healthy volunteers and 16 patients with CF. The inclusion criteria for both study groups consisted of 1) age >18 years, 2) free of obvious active tooth or gum disease, 3) no use of medication with antineutrophil or anti-inflammatory effect (aspirin, other NSAIDs, corticosteroids, and macrolide antibiotics) for 30 days before study, and 4) free of any illness for 14 days before study. Subjects with CF had to have a confirmed diagnosis based on sweat chloride greater than 60 mEq/l and typical pulmonary and/or gastrointestinal manifestations of CF. For both study groups, pregnant females; subjects with prior history of hypersensitivity to any NSAID; and subjects with significant history of hepatic, cardiovascular, renal, neurologic, hematologic, or peptic ulcer disease were excluded. The study was approved by the Institutional Review Board of University Hospitals of Cleveland. Informed consent was obtained from all subjects before study.

Study Design. The study period for each subject was 15 days and consisted of three periods defined as baseline (days 1, 2, and 3), treatment (days 3–12), and recovery (days 13, 14, and 15) (Fig. 1). Subjects were divided into five study groups based on the amount of ibuprofen to be received during the treatment period. Group 1 served as a control group (no ibuprofen) to establish the variability of oral mucosal PMN counts over the study period. The remaining four groups received ibuprofen, orally every 12 h, in open-label, at different doses during the treatment period. Group 2 received 2 to 3 mg/kg/dose, group 3 received 5 to 10 mg/kg, group 4 received 13 to 17 mg/kg, and group 5 received 20 to 30 mg/kg (maximum 3200 mg/day). The dose of ibuprofen was decreased by approximately 25% (on a milligram per kilogram basis) in the healthy subjects in an effort to achieve plasma concentrations similar to subjects with CF. The dose reduction was based on our previous comparison of ibuprofen pharmacokinetics in CF versus healthy subjects, where we observed that for a given dose, peak plasma concentration and area under the concentration-time curve were decreased, whereas apparent total clearance and apparent volume of distribution were increased in CF subjects compared with healthy subjects. Time to peak concentration and elimination half-life were similar (Konstan et al., 1991). As healthy and CF subjects were recruited, the first was assigned to group 1, the second to group 2, and so forth. After the fifth subject had been assigned to group 5, this pattern of assignment was repeated with subjects 6 to 10, and so on until all subjects were assigned to a study group. Subjects were allowed to participate more than once in the study, provided that a washout period of at least 1 month occurred.

During the baseline period, the subject visited the hospital on days 1, 2, and 3 to have an oral mucosal PMN count performed (measured from a 30-s mouthwash; procedure described below). On day 1, plasma was obtained for total ibuprofen and salicylic acid determinations. These determinations were done within 24 h, and any subject with either agent was to be excluded from further study (salicylic acid decreases the measured concentration of ibuprofen) (Albert and Gernaat, 1984).

The treatment period began immediately after the day 3 mouthwash (same morning), and the subject took the prescribed dose of ibuprofen (or none for those assigned to the control group). For those assigned to ibuprofen, a 10-h pharmacokinetic study to determine the peak plasma concentration ($C_{\text{max}}$) and area under the plasma concentration-time curve (AUC) was performed on this first dose (procedure described below). The subject returned home and took this same ibuprofen dose every 12 h (generally 9:00 AM and 9:00 PM) for the next 10 days. The subject returned to the hospital on days 8, 9, and 10 to have the mouthwash procedure repeated (test performed 12 h after taking ibuprofen), and the oral mucosal PMN count was determined for each day. The treatment period concluded on day 12 after the subject took the morning dose of ibuprofen.

During the recovery period, the subject returned to the hospital on days 13, 14, and 15 (morning) to have the mouthwash procedure repeated, and the oral mucosal PMN count was determined for each day. If by day 15 (72 h after discontinuing ibuprofen), the oral mucosal PMN count was not within 15% of the average count during the baseline period, the mouthwash procedure was scheduled to be repeated each morning until it returned to the baseline value.

In addition to the mouthwashes, all subjects had peripheral white cell blood counts with cell differentials performed once during the baseline (day 3), treatment (day 10), and recovery (day 15) period. These counts were used to determine whether the peripheral PMN pool remained stable during the study period. In addition to the ibuprofen and salicylic acid determinations on day 1 and the 10-h pharmacokinetic study of ibuprofen on day 3, plasma was obtained on day 10 (12 h after the previous dose) and day 13 (24 h after the last dose) for determination of these drugs (the plasma concentration of ibuprofen is generally not measurable, i.e., <0.5 μg/ml, in most individuals after 12 h). No subjects were found to have salicylate in their plasma.

Study Drug and Dosing Considerations. Ibuprofen, 200-mg tablets (Motrin), was supplied by The Upjohn Co. (Kalamazoo, MI). Ibuprofen doses ranged from 200 to 1,600 mg and were determined based on group assignment and body weight (range 2 to 30 mg/kg) as discussed under “Study Design”. As previously mentioned, the dose of ibuprofen was decreased by 25% in the healthy subjects in each group to achieve plasma concentrations similar to subjects with CF. The dose

![Fig. 1. Study design. The study period for each subject consisted of three periods defined as baseline (days 1, 2, and 3), treatment (days 3–12), and recovery (days 13, 14, and 15). Oral mucosal PMN counts were determined on three consecutive mornings during each period. The treatment period began immediately after the day 3 oral mucosal PMN count was determined, and the subject took the prescribed dose of ibuprofen every 12 h for 10 days (or none for those assigned to the control group). For those assigned to ibuprofen, a single 10-h pharmacokinetic study was performed on day 3 (●). The treatment period concluded on day 12 after the subject took the morning dose of ibuprofen. Peripheral white blood cell counts with cell differentials were obtained from each subject once during each period.](https://jpet.aspetjournals.org/content/1087/5/1087.f1)
ranges were chosen to target low (2–3 mg/kg), average (5–10 mg/kg), above average (15–17 mg/kg), and high-dose ibuprofen therapy (20–30 mg/kg). Low-dose therapy is often encountered by patients who self-medicate over-the-counter ibuprofen for treatment of headache or fever; average dose therapy is the recommended amount to be prescribed by physicians for the treatment of fever, and mild to moderate pain; above average doses are often prescribed for arthritis; and high-dose ibuprofen is occasionally used to treat arthritis and is the dose range in which we demonstrated that ibuprofen delays progression of lung disease in CF patients.

**Determining Oral Mucosal PMN Counts.** Oral mucosal PMN counts were determined using a modification of the mouthwash method of Wright et al. (1986). Normal saline (0.9% sodium chloride), 20 ml, was aliquoted into sterile cups. After an overnight fast (and withholding toothbrushing in the morning), the subject swished the saline in his or her mouth for 30 s, and returned the specimen to the cup. A second sample was obtained immediately after the first. The specimens were kept at 4°C and processed separately within 1 h of collection. The specimens were centrifuged at 1,000g at 4°C for 10 min, cell pellets were resuspended in 1 ml of acridine orange (3 μg/ml) (Sigma-Alrich, St. Louis, MO) in Hanks’ balanced salt solution (Invitrogen, Carlsbad, CA), and incubated at 37°C for 15 min in a shaking water bath. Because the acridine orange is phagocytosed by PMNs, PMNs are easily identified under fluorescent microscopy and counted with a hemocytometer. The total number of PMNs in each of the two specimens was recorded, the values were averaged, and the result expressed as total oral mucosal PMN counts for that study day.

**Ibuprofen Pharmacokinetics: Determining C_{\text{max}} and AUC.** The 10-h pharmacokinetic study was performed as follows. After an overnight fast and the completion of the day 3 mouthwash, a heparin lock was placed for venous access, and blood was obtained for ibuprofen analysis before and after study days in each period were averaged to establish the PMN count for each treatment period for each group. PMN counts from each of the three study days in each period were averaged to establish the PMN count for each period. The treatment PMN count was then expressed as a percentage of change from the baseline count. Although some subjects were present for the healthy subjects; no CF patient fell into the low range of PMN counts. To categorize subjects according to low, moderate, or high AUC, boundary values for AUC were determined based on a regression of AUC on C_{\text{max}}. The relationship between AUC and C_{\text{max}} from all subjects was modeled with a linear mixed model assuming a compound symmetry covariance structure using restricted maximum likelihood estimation.

To determine the duration of effect of ibuprofen on PMN migration, oral mucosal PMN counts during the recovery period (after ibuprofen had been discontinued) were compared with PMN counts from both the baseline and treatment periods for each group. This analysis also examined whether discontinuing high-dose ibuprofen was associated with an increase in PMN migration (rebound effect). A mixed linear model was used to estimate and test for within group changes from baseline using restricted maximum likelihood estimation implemented in SAS Proc Mixed (SAS Institute, Inc., Cary, NC) with time as a discrete factor. The covariance structure of the repeated measures for the group of patients not receiving ibuprofen was modeled using a compound symmetry model whereas a heterogeneous compound symmetry model was used for the group of subjects with peak ibuprofen plasma concentration >50 μg/ml. A similar analysis was performed on the peripheral white blood cell count data obtained during the baseline, treatment, and recovery periods. This analysis examined whether the number of peripheral white blood cells remained constant across the periods. All analyses were performed using SAS version 8.2.

**Results**

A total of 70 observations (47 healthy, 23 CF) were obtained from 51 subjects (35 healthy, 16 CF). Pharmacokinetic results are presented in Table 1. Studies are grouped based on peak plasma ibuprofen concentration, categorized as none, low C_{\text{max}} (<25 μg/ml), moderate C_{\text{max}} (25–50 μg/ml), and high C_{\text{max}} (>50 μg/ml). All of the groups were represented for the healthy subjects; no CF patient fell into the low C_{\text{max}} group. As expected, the broad dose range (low to high) achieved a broad range of peak concentrations.

The relationship between variable peak plasma ibuprofen concentrations (grouped by low, moderate, or high) and PMN migration to the oral mucosa is shown in Fig. 2. These results suggest that low peak ibuprofen concentrations are associated with an increase in PMN migration, whereas high peak concentrations (>50 μg/ml) are associated with a decrease in PMN migration. Even intermediate peak plasma concentrations (achieved by doses that are typically taken by individuals using over-the-counter or prescription ibuprofen for the relief of pain and fever) were associated with an increase in PMN migration. Similar findings were observed when the
subjects were categorized according to the AUC that was obtained (Fig. 3). The ranges for AUC correspond to the ranges for $C_{\text{max}}$ as determined by regression of AUC on $C_{\text{max}}$ (Fig. 4). Regression of AUC on $C_{\text{max}}$ yielded a predicted value of 11.0 mg·min/ml at a $C_{\text{max}}$ of 50 μg/ml, and 5.2 mg·min/ml at a $C_{\text{max}}$ of 25 μg/ml. The relationship between peak plasma ibuprofen concentration and PMN migration is shown in Fig. 5. In most cases (CF and healthy subjects), peak plasma concentration >50 μg/ml was associated with a decrease in PMN migration [mean change $-27.3 \pm 4.6$ (S.E.M.) %, $p < 0.001$]. Subjects for whom ibuprofen at $C_{\text{max}}$ >50 μg/ml was not associated with decreased PMN migration had lower AUCs (<11.0). A concentration-response above 50 μg/ml did not occur ($C_{\text{max}}$ much greater than 50 μg/ml was not associated with a greater decrease in PMN migration than $C_{\text{max}}$ of 50 μg/ml). Ibuprofen peak concentrations <50 μg/ml in most cases was associated with an increase in PMN migration (mean change $+39.4 \pm 6.3$ %, $p < 0.001$). The relationship between peak plasma ibuprofen concentration and PMN migration in CF was similar to healthy subjects ($p = 0.93$).

The relationship between AUC for ibuprofen and PMN migration is shown in Fig. 6. All subjects with AUC >11.0 had a decrease in PMN migration (mean change $-32.7 \pm 2.2$ %, $p < 0.001$), whereas those with AUC <11.0 had an increase in PMN migration of 32.6 ± 6.7 %, $p < 0.001$.

The duration of effect of ibuprofen (50–90 μg/ml) on PMN delivery to the oral mucosa is shown in Fig. 7. A consistent decrease in PMN migration was present 12 h after dosing. However, 24 h after a dose, PMN migration had returned to baseline levels. Migration did not significantly exceed baseline levels at any time point (24–72 h) after dosing, indicating little or no “rebound” effect. Peripheral white blood cell counts and absolute neutrophil counts remained stable over the course of study in each of the four $C_{\text{max}}$ groups of subjects (data not shown). Thus, the changes in oral mucosal PMN counts observed in the different $C_{\text{max}}$ groups cannot be explained by an effect of ibuprofen on the peripheral blood PMN count.

**Discussion**

In patients with cystic fibrosis, there is a massive and persistent influx of neutrophils into the airways, which ultimately destroys the lungs (Konstan and Berger, 1993, 1997). Therefore, strategies for decreasing neutrophil influx or countering neutrophil products should be beneficial (Konstan, 1996). In a 4-year clinical trial in patients with CF,
twice daily administration of ibuprofen retarded the progression of lung disease (Konstan et al., 1995). The rationale for using ibuprofen in this trial was based on its antineutrophil properties (Brown and Collins, 1977; Spisani et al., 1978; Higgs et al., 1980; Flynn et al., 1984; Kaplan et al., 1984; Maderazo et al., 1984; Shimanuki et al., 1985; Venezio et al., 1985), and the demonstration that ibuprofen decreased PMN influx in several animal models of pulmonary infection and inflammation (Sordelli et al., 1985; Rinaldo and Pennock, 1986). Interestingly, one of these models revealed that low-dose ibuprofen (3 mg/kg) resulted in increased PMN influx, whereas in this same model, high-dose ibuprofen (30 mg/kg) decreased PMN influx (Rinaldo and Pennock, 1986). This dose effect has important implications for the treatment of conditions in which neutrophil-dominated inflammation plays a major role. If a similar response occurs in humans, an increase in PMN delivery to the lung after low-dose ibuprofen therapy to a patient with neutrophil-mediated lung disease would likely hasten lung destruction.

Therefore, careful consideration of dosing and frequency of dosing was made during the design of the 4-year trial of ibuprofen in CF patients to assure that ibuprofen would limit PMN...
migration rather than promote it. In that trial, ibuprofen doses were adjusted by pharmacokinetic studies in each patient to maintain a peak plasma concentration between 50 and 100 \( \mu g/ml \) throughout the trial (Konstan et al., 1995). Ibuprofen is now advocated as a treatment for CF lung disease, with the strong recommendation that concentration monitoring be performed to ensure that peak plasma concentrations are maintained between 50 and 100 \( \mu g/ml \) (Konstan, 1996).

Although the presumed beneficial effect of ibuprofen on CF lung disease is due to a decrease in PMN influx, this has not been demonstrated. Although it would be desirable to assess the effect of ibuprofen on PMN migration to the lung itself in patients with CF, this would require serial bronchoalveolar lavage, which would not be a practical way to establish dose-response or concentration-response curves, or ultimately to adjust doses in an individual patient. Therefore, we assessed the effect of ibuprofen on PMN migration to a mucosal surface outside of the lung, the oral mucosa, using the sensitive and reproducible mouthwash method (Wright et al., 1986). The inhibitory effect of corticosteroids on PMN delivery to tissues has been demonstrated with this technique (Wright et al., 1986). Although it is possible that organ-specific factors in the lung, particularly the CF lung, could result in a different effect of ibuprofen, no better surrogate for the mucosa of the lower airway exists for the evaluation of neutrophil delivery to a mucosal surface.

In the present study, we found that a peak plasma ibuprofen concentration >50 \( \mu g/ml \) was required to decrease PMN migration to a mucosal surface in healthy and CF individuals. Peak plasma concentrations much greater than 50 \( \mu g/ml \) did not result in a greater decrease in PMN migration. In fact, it is notable that there was not a concentration-dependent response in the group of subjects who achieved peak plasma concentrations >50 \( \mu g/ml \). Peak plasma concentrations below 50 \( \mu g/ml \) increased PMN migration in both healthy and CF individuals. These results are consistent with there being a threshold effect of ibuprofen on PMN migration (a transition point from where ibuprofen promotes PMN migration to where it limits PMN migration), but not a concentration effect after the threshold has been achieved. Therefore, this study supports the recommendation that peak plasma concentrations >50 \( \mu g/ml \) need to be maintained in patients with CF, and further suggests that higher concentrations do not confer additional benefit. Similar relationships were observed for AUC and PMN migration. However, because of the design of this study, we did not formally test whether \( C_{max} \) or AUC is a better predictor of PMN migration. The threshold AUC for decreasing PMN migration in both healthy and CF individuals was 11.0 mg-min/ml. However, AUC assessment is not recommended for the clinical monitoring of ibuprofen in CF, because it requires an 8- to 10-h pharmacokinetic study as opposed to a 3-h study. Hourly sampling for 3 h is sufficient to assess for \( C_{max} \) in most individuals (Konstan et al., 1991); in this study, 90\% of the subjects achieved \( C_{max} \) by 3 h. A dosing interval of 12 h was required to achieve the desired effect on PMN migration (24 h was too long). Thus, the current recommendation of twice daily dosing appears to be valid. There was no rebound effect (increased PMN migration) after discontinuing ibuprofen. This result should help assure clinicians that discontinuing ibuprofen therapy during the administration of nephrotoxic antibiotics for the treatment of pulmonary exacerbations is not harmful.

Although this study provides considerable useful information, it has limitations. The relationship between PMN migration to oral mucosa and to the lung has not been established, and the adequacy of this model system as a surrogate marker is not established. In addition, we did not systematically vary AUC independent of \( C_{max} \), a study that would be of interest. Shorter dosing intervals with lower individual doses or alternative NSAIDs were not explored. Future studies could examine these questions. However, this study suggests that twice daily dosing of ibuprofen to achieve \( C_{max} \) >50 \( \mu g/ml \) will reduce PMN migration to an inflammatory site, and this is a practical regimen for long-term use.

Overall, these results suggest that careful and sustained dosing of CF patients with ibuprofen is required to achieve the desired effect and to minimize adverse effects of ibuprofen on PMN migration. Although this study was directed toward an assessment of the use of ibuprofen for the treatment of CF lung disease, the information gained from this study could prove useful for a number of other conditions in which ibuprofen is used to treat inflammation, including rheumatoid arthritis, where neutrophils play a prominent role.

References

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