Kolkman-Pena.qxd 21/09/04 12:51 PÆgina 589

Drugs of Today 2004, 40 (7): 589-601
Copyright © 2004 PROUS SCIENCE
CCC: 0025-7656/2004

EVALUATION OF ORAL BUDESONIDE IN THE TREATMENT OF ACTIVE DISTAL ULCERATIVE COLITIS

Jeroen J. Kolkman[1], Helmut W. Möllmann[2], Anja Clara Möllmann[3], Amado Salvador Peña[4], Roland Greinwald[5], Horst-Dietmar Tauschel[5] and Guenther Hochhaus[3]

[1]Medisch Spectrum Twente, Enschede, the Netherlands; [2]Medizinische Klinik BG-Kliniken Bergmannsheil, Universitätsklinik, Bochum, Germany; [3]Department of Pharmaceutics, College of Pharmacy, J. Hillis Miller Health Center, Gainesville, Florida, USA; [4]Vrije Universiteit Medical Centre, Amsterdam, the Netherlands; [5]Dr. Falk Pharma GmbH, Freiburg, Germany

CONTENTS

Summary 589
Introduction. 590
Material and methods 591
Patients and protocol 591
Pharmacokinetics and analytical methods. 591
Other pharmacodynamic parameters 593
Clinical and endoscopic efficacy 593
Results 594
Pharmacokinetics 594
Pharmacodynamics 595
Efficacy data and safety 595
Discussion 598
References 600

Summary

Budesonide, a topical corticosteroid, has proven useful for the management of Crohn's disease. Its efficacy is similar to prednisone but it has fewer side effects. A new pH-modified release capsule (*Budenofalk®*) is probably efficacious in distal ulcerative colitis. The aim of the present study was to establish the pharmacokinetics, pharmacodynamics, and safety of two dosage regimens of budesonide capsules and to obtain efficacy information. *Budenofalk®* 9 mg daily was administered as a single dose 9 mg in 8 patients and as three 3 mg doses in 7 patients with active distal ulcerative colitis for 8 weeks. Symptoms were assessed at three timepoints during the study: baseline, 4 and 8 weeks af-

Correspondence: Prof. A.S. Peña MD, PhD, FRCP, Department of Gastroenterology, Head, Laboratory of Immunogenetics, VU University Medical Center, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands. Tel.: +31 20 444 8416; FAX: +31 20 444 8418; E-mail: as.pena@vumc.nl. Web site: http://www.immunogenetics.nl/

Dr. Falk Pharma Ex. 1011
IPR Petition - USP 11,260,061

Kolkman-Pena.qxd 21/09/04 12:51 PÆgina 590

ter start of treatment. Endoscopic evaluation and budesonide concentration in mucosal biopsy specimens was performed at 0 and 8 weeks. A pharmacokinetic profile and pharmacodynamic profile (cortisol, lymphocytes and neutrophils) was performed at day 5. In the 9 mg o.d. group, higher peak concentrations and systemic availability was found compared to the 3 mg t.i.d. group. Cortisol suppression was more pronounced after 9 mg o.d. than after 3 mg t.i.d. Lag-time, AUC and pharmacodynamic effects were comparable (14% mean decrease lymphocyte count and 26% mean increase neutrophil count). Mucosal biopsy specimens in the distal colon revealed significant budesonide levels with both regimens. After 8 weeks, 71% in the 9 mg o.d. group and 38% in the 3 t.i.d. group responded. The endoscopic index improved from 10 ± 2 to 2 ± 3 (p <0.001) with 9 mg o.d. and from 9 ± 2 to 4 ± 4.7 (p = 0.02) with 3 mg t.i.d. The pharmacokinetic and pharmacodynamic profiles found in this study indicate that *Budenofalk®* reaches the distal part of colon and rectum, but further studies to validate the budesonide assay in the mucosa and comparison with a control group are necessary. This limited study suggests that *Budenofalk®* is effective in distal colitis and side effects are rare. Based on these observations a large clinical trial using 9 mg o.d. is indicated to confirm efficacy and assess further possible side effects. © 2004 Prous Science. All rights reserved.

Introduction

Systemically administered glucocorticoids are effective in the treatment of ulcerative colitis, however, their use is hampered by the frequent occurrence of systemic side effects and the related "steroid" fear of patients, which often result in reduced compliance. The increasingly used topical glucocorticosteroids are characterized by drug release in the diseased area, resulting in high drug concentrations and fewer systemic effects. Moreover, these drugs are characterized by low oral bioavailability and efficient removal from the systemic circulation, leading to fewer systemic side effects.

For the past 20 years, budesonide, a prototype of a topically acting glucocorticoid, has been used for the treatment of asthma and other diseases of the respiratory tract. In addition, budesonide is also used for the treatment of Crohn's disease and ulcerative colitis (1–3) when given in formulations that insure that the drug is released at high concentrations at the disease area (4).

Budenofalk® pH-modified release capsules, composed of budesonide pellets coated with methacrylic polymers (4), fulfill this criterion: drug release is prevented in the stomach and proximal small bowel but occurs in a pH-dependent manner in the intestine and colon. About 350 of these pellets (representing a total dose of 3 mg budesonide) are placed into gastric juice-soluble hard gelatine capsules. The pellets, released in the stomach, are able to pass the pylorus intact and independent of the interdigestive phase III (due to their small diameter). They subsequently enter the intestine, and the polymer coating starts degrading at pH 6.4, releasing the drug in the ileum and ascending colon. This delivery system has been shown to improve clinical and functional outcomes as quickly and efficiently as systemically acting glucocorticoids such as prednisone in patients with active Crohn's disease (5–8).

Two controlled, double-blind, dose-finding studies have shown that 9 mg of oral budesonide per day was the most effective dose in the treatment of active Crohn's disease (8, 9). Other studies demonstrated that budesonide and prednisolone/prednisone are therapeutically equivalent in the treatment of active Crohn's disease, but that budesonide induces fewer glucocorticoid-related side effects and less suppression of the pituitary-adrenal function (5, 6). The efficacy of oral budesonide in active Crohn's disease is well documented (3), however only preliminary data are available for its efficacy in the treatment of ulcerative colitis (10).

A pilot study suggested that the administration of pH-modified release budesonide capsules had a therapeutic effect on the activity of steroid-dependent ulcerative colitis and exerted a significant steroid-sparing effect (11). Beneficial effects were demonstrated in patients exhibiting steroid-dependent ulcerative colitis when the new oral budesonide formulation was combined with budesonide enemas (12, 13). In an open clinical trial with 72 patients, the effect of oral budesonide in patients with active ulcerative colitis was compared to that of prednisolone. The results in the two treatment groups were comparable, but in contrast to prednisolone, budesonide did not change the plasma cortisol level (10). A case-report of a 14-year-old girl suffering from steroid-dependent chronic, active, severe ulcerative colitis demonstrated complete remission after changing from prednisolone to oral budesonide 3 mg t.i.d. Moreover, the progression of osteopenia and growth retardation caused by the prednisolone treatment was stopped (14).

Dr. Falk Pharma Ex. 1011
IPR Petition - USP 11,260,061

Kolkman-Pena.qxd 21/09/04 12:51 PÆgina 591

Previous reports described the pharmacokinetics and pharmacodynamics of single (1 x 3 mg) and multiple (3 x 3 mg) budesonide dosage regimens of 3 mg capsules in healthy volunteers (15).

The aim of the present study was to investigate the pharmacokinetics and pharmacodynamics of topically acting budesonide in patients with mild to moderately active, left-sided ulcerative colitis using the dosage regimens of 3 x 3 mg or 1 x 9 mg budesonide per day. The two dosing regimens were used in order to evaluate whether or not a single high daily dose exhibited greater efficacy than the same dose administered three times throughout the day. This was of interest as pharmacokinetic/pharmacodynamic modeling approaches suggested a higher efficacy of the divided dosing regimens. In addition, the budesonide concentrations in biopsy specimens of the rectum, sigmoid and descending colon before treatment and at steady state were assessed to determine whether pharmacologically relevant concentrations were measurable at the site of action. A further secondary objective was to compare surrogate markers of systemic side effects, such as serum cortisol and blood lymphocytes and granulocytes, in order to allow the assessment of the safety of the two dosing regimens and to obtain information on clinical efficacy (monitored by the clinical activity index [CAI]).

Material and methods

Patients and protocol

Male and female patients with mild to moderate, left-sided active proctitis, proctosigmoiditis or left-sided ulcerative colitis were included in the study after diagnosis was confirmed by colonoscopy and histology (Table I). Qualified patients had CAI score > 4 and EI (endoscopic index) = 4. Patients were not allowed to receive concomitant active medication for ulcerative colitis such as glucocorticoids or immunosuppressants.

In group A, receiving 3 x 3 mg budesonide per day, the 8 patients (6 females, 2 males) with ulcerative colitis included in the evaluation were 30.4 ± 16.54 years old (range: 17–67 years), had a body weight of 66.5 ± 13.73 kg (range: 48–79 kg) and an average height of 172.9 ± 10.78 cm (range: 158–190 cm).They were all Caucasian. In group B, receiving 1 x 9 mg budesonide per day, the 7 patients (4 females, 3 males) were 48.6 ± 16.38 years old (range: 27–69 years), had a body weight of 81.6 ± 17.64 kg (range: 56–107 kg) and an average height of 170.6 ± 9.07 cm (range: 163–182 cm). Four patients were Caucasian, two were of African origin and one patient was Lebanese.

The present study was conducted as a controlled, multicenter, randomized, open phase II clinical trial. Patients were randomly assigned either to group A or group B for 8 weeks, or for 4 weeks in case of remission. On study day 5, pharmacokinetic (budesonide concentrations) and pharmacodynamic parameters (cortisol as well as blood lymphocytes and neutrophils as alternative markers of systemic effects) were assessed over 24 hours for both dosage regimens of budesonide capsules. In addition, the budesonide concentration was determined in biopsy material of the descending colon (± 50 cm), the sigmoid colon (± 25 cm) and the rectum (± 10 cm) at baseline (day 0) and on day 56 of treatment. The CAI was determined at baseline and after 4 and 8 weeks of treatment. The EI was determined at baseline and after 8 weeks, or after 4 weeks if the patient was in remission and treatment was discontinued. Both activity indices were determined according to Rachmilewitz (16). Furthermore, the number of stools per week, the percentage of days with bloody stools per week as well as C-reactive protein concentrations and the blood sedimentation rate (ESR) were regularly documented throughout the study period.

Pharmacokinetics and analytical methods

The purpose of this study was to investigate the pharmacokinetics after 3 x 3 mg and 1 x 9 mg daily doses of 3 mg budesonide capsules at steady state in patients with acute, left-sided ulcerative colitis. The study was designed to allow an optimal assessment of the pharmacokinetic properties.

The patients enrolled in this study were hospitalized on the evening of day 4 prior to the pharmacokinetic analysis on days 5 and 6. They remained in the hospital until the morning of day 6 for the last blood withdrawal at 7:00 a.m. The patients in group A ingested the evening dose on day 4 at 7:00 p.m. On day 5 group A ingested the capsules in a 5 hour-interval, starting at 7:00 a.m. Group B ingested the single dose of 9 mg at 7:00 a.m.

Seventeen blood samples of 5 ml each were drawn from both groups A and B to determine the budesonide and cortisol concentrations in serum. The first blood sample was drawn at 7.00 a.m. immediately before capsule ingestion and after at least 10 hours of fasting. After ingesting the morning dose, additional blood samples were taken at 1, 2, 3, 4, 5, 6, 8, 9, 10, 12, 14, 15, 16, 18, 20 and

Dr. Falk Pharma Ex. 1011
IPR Petition - USP 11,260,061

Kolkman-Pena.qxd 21/09/04 12:51 Página 592

Table I: Baseline demography and characteristics of patients with ulcerative colitis treated with 3 x 3 mg or 1 x 9 mg budesonide daily.

Group A: 3 x 3 mg, 8 patients with UC:

Subj. code	Subj. initials	Age (years)	Sex	Location of UC: Rectum	Location of UC: Procto-sigmoid	Location of UC: Higher	Risk factors: Non-smoker	Risk factors: Ex-smoker	Risk factors: Smoker	Years since first attack	Duration of present episode (days)	Stools/ week	Bloody stools/ week (%)	CRP (mg/l) day 5	ESR (%) day 5	EI	CAI	Additional medication
1	RMD	25	M		X							18	100	9.0	<50	≥4	>4	Mesalazine
4	EKB	40	F		X							29	100	9.0	<50	≥4	>4	–
17	HN	29	F		X							51	100	50.0	<50	≥4	>4	Mesalazine
21	AF	18	F		X							24	80	1.0	<50	≥4	>4	Mesalazine
22	KB	27	F		X							47	15	13.0	<50	≥4	>4	Mesalazine
25	LVL	20	F		X							23	100	9.9	<50	≥4	>4	–
27	JBO	18	M	X								35	57	28.8	<50	≥4	>4	–
29	ABD	67	F		X							12	100	9.9	<50	≥4	>4	Mesalazine
Mean		30.5	2M (25%)	1 (13%)	7(87%)	0(0%)	7(88%)	1(12%)	0(0%)	11.9	79.4					8.8	7.1	
SD		16.5	6F (75%)															

Group B: 1 x 9 mg, 7 patients with UC:

Subj. code	Subj. initials	Age (years)	Sex	Location of UC: Rectum	Location of UC: Procto-sigmoid	Location of UC: Higher	Risk factors: Non-smoker	Risk factors: Ex-smoker	Risk factors: Smoker	Years since first attack	Duration of present episode (days)	Stools/ week	Bloody stools/ week (%)	CRP (mg/l) day 5	ESR (%) day 5	EI	CAI	Additional medication
2	JHA	52	M		X							28	71	9.0	<50	≥4	>4	–
3	EPI	27	F	X								18	86	9.0	<50	≥4	>4	–
23	KL	69	F			X						15	100	1.0	<50	≥4	>4	Mesalazine
26	ICH	42	M		X							23	100	9.0	<50	≥4	>4	–
28	KKR	29	F		X							39	100	9.0	<50	≥4	>4	Mesalazine
30	GNI	59	M		X							18	100	20.0	<50	≥4	>4	Mesalazine
31	IMZ	62	F		X							49	100	9.9	<50	≥4	>4	Mesalazine
Mean		48.6	3M (43%)	1 (14%)	5(72%)	1(14%)	4(57%)	2(29%)	1(14%)	10.7	66.1					9.7	6.9	
SD		16.4	4F (57%)															

UC: ulcerative colitis, EI: endoscopic index at baseline, CAI: clinical activity index at baseline.
CRP, ESR, Cortisol, lymphocytes, neutrophils on day 5/6 over 24 hours.
Stools/week and % bloody stools/week at baseline.

Dr. Falk Pharma Ex. 1011
IPR Petition - USP 11,260,061

Kolkman-Pena.qxd 21/09/04 12:51 PÆgina 593

24 hours (the last was taken at 7:00 a.m. on day 6). Serum was separated from the 5 ml venous blood samples by centrifugation, and immediately frozen and stored at –20 °C until analyzed.

On day 0 and day 56 of the clinical trial period, biopsies were taken from the descending colon, the sigmoid colon and rectum, immediately frozen and stored at –20 °C. Often, two biopsy samples were provided for a given region.

Budesonide concentrations in serum were determined using a validated HPLC/MS/MS assay with a sensitivity of 50 pg/ml (17).

Budesonide levels in biopsy material were determined by HPLC/MS after homogenization in saline, liquid/liquid extraction followed by solid phase extraction, derivatization and HPLC/MS, similar to the method described for serum (17). The assay could not be validated, as blank biopsy material was not available for comparison. Applying the assay performance of the serum assay, and considering the much smaller amount of biopsy material available, a sensitivity of about 5–10 ng/g of wet tissue was indicated.

The pharmacokinetic analysis was performed using noncompartmental approaches using *Kinetica*® software (Version 3.1, InnaPhase Corporation, Philadelphia, PA). Data below the limit of detection were not considered.

The following parameters were evaluated: time to peak serum concentration (T_{max}) and peak concentration (C_{max}) were obtained directly from the concentration *versus* time data. The time-lag of absorption (T_{lag}) was defined as the time after drug administration at which concentrations increased for the first time above baseline values. The area under the concentration-time curve between 0 hours (day 5) and the last measurement point after 24 hours (AUC_{0-24}) was calculated by the trapezoidal rule. The terminal elimination rate constant (K_e) was determined for each individual subject from the terminal slopes of semi-logarithmic plots of serum concentration-time profiles. For the 3 x 3 mg dosing regimen, the window of time was generally 12–24 hours. The elimination half-life was calculated as $t_{1/2} = 0.693/K_e$.

Apparent clearance (Cl) not adjusted for the systemic bioavailability (f) was calculated from AUC_{0-24} and the administered dose (= 9 mg).

Other pharmacodynamic parameters

Neutrophils (% of leucocytes) and lymphocytes (% of leucocytes) were used as pharmacodynamic parameters, beside cortisol, to describe the systemic effects. Data analysis was based on relative cell numbers (% of total white blood cells), and performed after transformation of the data into percent of baseline estimates. As a cumulative measure for the pharmacodynamic activity on blood cells, the area under the effect parameter-time curve was calculated by the trapezoidal rule over 24 hours. Missing data points were substituted with the group means for the specific time point. Lymphocyte and neutrophil changes were calculated individually from the AUC_{0-24} by calculating the deviation from the zero-time estimate (100%, equivalent to a 24-hour AUC of 2400 % x h^{-1}) as:

$$\% \text{ Change} = (2400 - AUC) \times 100/2400 \% \times h^{-1}$$

Thus, the obtained percent change was positive for lymphocytes and negative for the effects on neutrophils. These calculations assumed that the baseline value at 7 a.m. was not modulated by remaining budesonide.

Serum cortisol concentrations were also used as pharmacodynamic parameters describing the systemic effects. The AUC_{0-24} was calculated by the trapezoidal rule over 24 hours as a cumulative measure for the pharmacodynamic activity. Missing data points were substituted for with the estimates of the group means for the specific time point.

Serum cortisol concentrations were determined with a competitive solid-phase radioimmunoassay, applicable for measurements in serum (Coat-A-Count, Diagnostic Products Corp., obtained through H. Biermann GmbH, Bad Nauheim, FRG). The samples (25 l of serum) were incubated for 45 min with ^{125}I-labled cortisol in tubes coated with anticortisol antibodies. After aspirating the liquid from each tube, the radioactivity in the tubes was measured with a gamma counter (Multi-Crystal Counter LB 2104; Berthold, Bad Wildbad, FRG). The concentrations of the samples were computed from standard curves (linear-log transformation). The between–assay's coefficient of variation was 6.3% in serum at a mean cortisol concentration of 48 g/l. Cortisol standards for serum assays were used in concentration ranges of 10–500 g/l. The cross reactivity of the assay for cortisone, cortisol acetate and cortisone acetate was < 1.0%, 4.7% and < 1.0%, respectively.

Clinical and endoscopic efficacy

Clinical efficacy was defined as CAI $\leq$ 4 (clinical remission as defined by Rachmilewitz [16] at day 28 [second control] and day 56 [final control]). Non-

Dr. Falk Pharma Ex. 1011
IPR Petition - USP 11,260,061

Kolkman-Pena.qxd 21/09/04 12:51 PÆgina 594

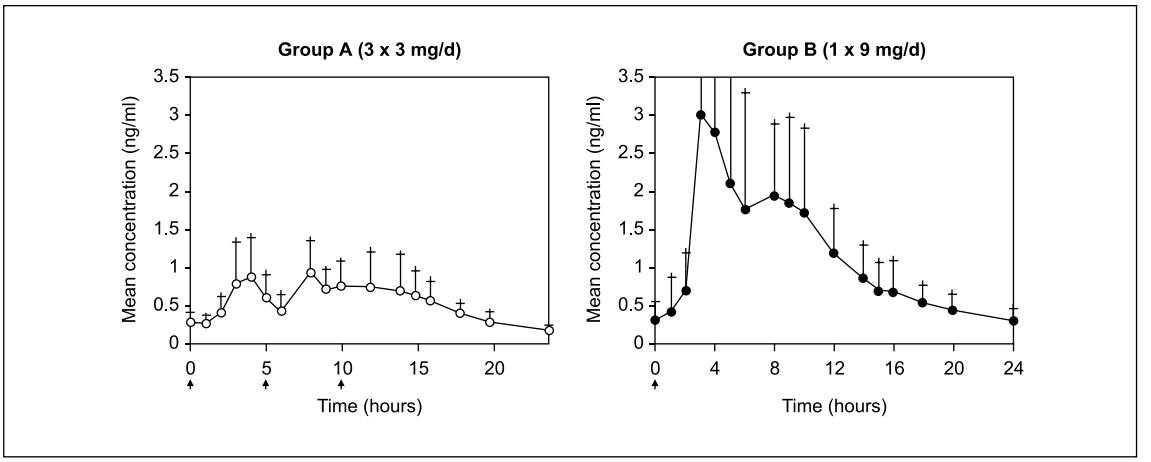

Fig. 1. Mean serum concentrations (± SD) after the daily administration of 3 x 3 mg (group A) and 1 x 9 mg (group B) budesonide daily in patients with ulcerative colitis on day 5 of the treatment. Evaluation performed by the HPLC/MS/MS method.

response was defined as CAI > 4 (absence of clinical remission) at the same time. Patients who were not in remission and had discontinued therapy on day 24 or earlier were considered to be nonresponders at both times of assessment. Patients who were not in remission and had stopped therapy before day 49, but after day 24, were evaluated as nonresponders at the final visit. Clinical improvement was defined as a decrease in CAI greater than 30% at day 28 or day 56, respectively, compared to the baseline value. Endoscopic remission was defined as EI < 4, evaluated at final control after 8 weeks, or after 4 weeks if the patient was in remission and treatment was discontinued.

Results

Pharmacokinetics

Eight patients in group A and 7 patients in group B finished the pharmacokinetic part of the study. One patient in group A was not included in the analysis, as this patient received budesonide every 6 hours. Another patient in this group was not included in the analysis because of study deviations (age = 17 years and failure to take study medication for 3 days). Serum samples of one patient were not available. Thus, 6 patients of group A and 7 patients of group B were included in the pharmacokinetic analysis.

Mean serum budesonide time profiles for groups A and B are presented in Figure 1. Mean calculated pharmacokinetic parameters for budesonide are shown in Table II. As to be expected for a comparison of single *versus* three-times daily dosing, absorption profiles differed between the two dosing regimens with higher peak levels observed for the 1 x 9 mg dosing. However, in addition to individual dose-related differences, the serum levels allowed additional distinct pharmacokinetic observations (Table II). A statistically later T_{max} value observed for the 1 x 9 mg treatment (5.4 h *vs.* 3.4 h for the 3 x 3 mg treatment) is certainly due to the higher single-dose regime. In addition, the two groups differed in K_e, $t_{1/2}$, AUC and Cl/f. These differences were significant at the $p < 0.05$ level. Calculated ap-

Table II: Mean (± SD) pharmacokinetic parameters for budesonide under steady-state conditions on day 5 of dosing after administration of 3 x 3 mg (group A) or 1 x 9 mg (group B) budesonide once daily in patients with ulcerative colitis. Analysis with HPLC/MS/MS method.

Parameter	Group A (3 x 3 mg)	Group B (1 x 9 mg)
n	6	7
T_{lag} (h)	1.8 ± 0.8	1.3 ± 0.8
$C_{max,1}$ (ng/ml)	0.98 ± 0.66	4.10 ± 2.53
$C_{max,2}$ (ng/ml)	1.03 ± 0.48	
$C_{max,3}$ (ng/ml)	0.80 ± 0.55	
$T_{max,1}$ (h)	3.4 ± 0.5	5.4 ± 2.8
$T_{max,2}$ (h)	3.4 ± 0.9	
$T_{max,3}$ (h)	4.4 ± 1.5	
$AUC_{0\text{-last}}$ (ng•h/ml)	13.14 ± 6.24	27.6 ± 14.9
k_e (h^{-1})	0.176 ± 0.059	0.096 ± 0.040
$t_{½}$ (h)	4.3 ± 1.4	8.4 ± 3.8
Cl/f (l/h)	14.39 ± 8.46	7.38 ± 4.7

Dr. Falk Pharma Ex. 1011
IPR Petition - USP 11,260,061

Kolkman-Pena.qxd 21/09/04 12:51 PÆgina 595

Table III: Average budesonide concentrations in biopsies of group A and group B observed on day 0 and day 56 after administration of 3 x 3 mg and 1 x 9 mg budesonide, respectively, in patients with ulcerative colitis.

	Group A (3 x 3 mg)		Group B (1 x 9 mg)	
Day 0	Mean ng/g	± SD	Mean ng/g	± SD
Descending Colon	23.6	51.5	4.1	4.3
Sigmoid	9.2	14.0	4.2	7.1
Rectum	17.8	19.6	14.5	19.5
Day 56	Mean ng/g	± SD	Mean ng/g	± SD
Descending colon	59.4	74.5	23.0	7.4
Sigmoid	15.5	5.2	54.5	84.7
Rectum	47.3	32.0	47.9	22.7

parent Cl/f values are consistent with the apparent increased bioavailability of a single 9 mg dose compared to administration in divided doses. Using these estimates and the clearance of budesonide in adults with active Crohn's disease (61.2 l/h) (18), the oral bioavailability was determined to be 7% and 14% for the 3 x 3 and 1 x 9 mg regimens, respectively.

Concentrations measured in biopsy specimens obtained on day 0 and day 56 of the clinical trial are given in Table III and graphed in Figure 2. In some patients, two biopsy samples were provided for a given colon or rectum region. Comparison of these individual data suggested a distinct variability of the assay, especially when drug levels were close to the suggested limit of quantification (5–10 ng/ml). A potential reason might be interference of the assay with endogenous substances. This is also indicated by the fact that on day 0, nine out of 54 samples showed budesonide levels distinctly different from the suggested limit of detection (larger than 20 ng/g considering a limit of detection of 5–10 ng/g). Such interferences could not be excluded because of lack of sufficient "placebo" biopsy specimens for validation of the assay. The results, however, show a tendency of increased budesonide levels after treatment, with no distinct differences between regional concentrations and dosing regimens when the distinct variability in the concentrations is considered (Table III).

Pharmacodynamics

Systemic pharmacodynamic effects were assessed by monitoring the effects of budesonide on lymphocytes, neutrophils and cortisol.

Lymphocytes and neutrophils

The mean changes of blood lymphocyte and neutrophil counts, based on baseline values and calculated from the AUC_{0-24} obtained after application of 3 x 3 mg budesonide or 1 x 9 mg budesonide on day 5–6 of treatment, are shown in Figure 3 and Table IV. While the number of lymphocytes slightly decreased in group A and group B (11.9% and

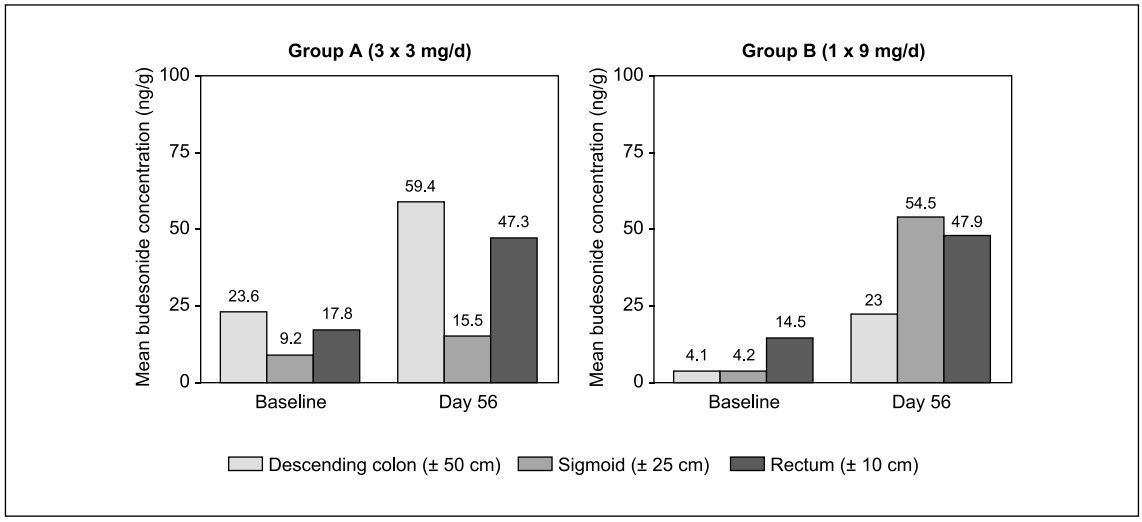

Fig. 2. Budesonide mean concentration. Biopsies in the descending colon, sigmoid and rectum in patients with ulcerative colitis after administration of 3 x 3 mg (group A) and 1 x 9 mg (group B) budesonide at baseline and on day 56.

Dr. Falk Pharma Ex. 1011
IPR Petition - USP 11,260,061

Kolkman-Pena.qxd 21/09/04 12:51 PÆgina 596

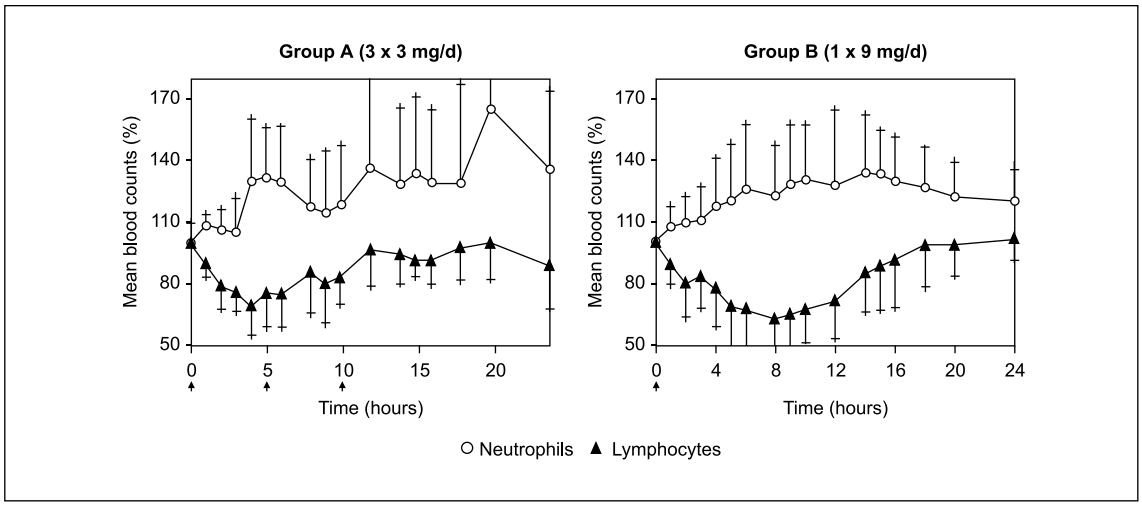

Fig. 3. Mean (± SD) blood neutrophil and lymphocyte counts (% of pre-dose level based on relative cell numbers) after the daily administration of 3 x 3 mg (group A) and 1 x 9 mg (group B) budesonide in patients with ulcerative colitis on day 5 of treatment.

Table IV: Changes in blood lymphocyte and neutrophil counts based on baseline estimates and calculated from the area under the effect parameter time profile over 24 hours obtained after application of 3 x 3 mg (group A) or 1 x 9 mg (group B) budesonide on day 5 of treatment.

Parameter	Budesonide dosage	% Change Mean	± S.D.	*p*-value
Lymphocytes	3 x 3 mg	–11.9	8.0	
	1 x 9 mg	–16.8	14.7	0.242
Neutrophils	3 x 3 mg	28.9	31.2	
	1 x 9 mg	23.1	17.2	0.341

Table V: Area under the 24-hour serum cortisol concentration-time profile (mean ± SD) after oral administration of 3 x 3 mg (group A) or 1 x 9 mg (group B) budesonide in patients with ulcerative colitis at steady state (day 5).

Group	Cortisol AUC $_{0–24\ h}$ (ng•h/ml) Mean	± S.D.	*p*-value
Group A (3 x 3 mg budesonide)	1926.3	741.3	
Group B (1 x 9 mg budesonide)	1085.5	443.5	0.0196

16.8%, respectively), the increase in neutrophils was more pronounced (28.9% and 23.1%, respectively). A *t*-test analysis of lymphocyte and neutrophil AUC_{0-24} values did not indicate a significant difference between group A and group B.

Serum cortisol

The endogenous 24-hour cortisol was monitored for both treatment groups after administration of budesonide on day 5. Figure 4 shows that cortisol suppression was somewhat more pronounced after 1 x 9 mg budesonide (group B) than after 3 x 3 mg budesonide (group A). A *t*-test analysis of the serum cortisol AUC revealed that the cumulative cortisol level was significantly lower for group B than for group A (Table V).

Efficacy data and safety

Only patients with active ulcerative colitis (CAI >4) distal to the splenic flexure were included for the assessment of the CAI. After 4 weeks, complete response (CAI ≤4) was reached in 0 patients in group A, but in 3 patients in group B. The overall response rate (CAI ≤4 or 30% decrease in CAI) after 8 weeks was greater with 1 x 9 mg (71%) compared to 3 x 3 mg (38%) (Table VI) (Fig. 5). The number of stools per day at day 0 and 56 did not change in the 3 x 3 mg group (median 3.8, range 1–6, and median 3.0, range 1–11, respectively), but decreased from median 3.4 (range 2–8) to 1.9 (range 1–8, $p = 0.02$) in the 1 x 9 mg group. The percentage of bloody stools reduced in both groups: from median 100% (range 29–100%) to median 29%

Dr. Falk Pharma Ex. 1011
IPR Petition - USP 11,260,061

Kolkman-Pena.qxd 21/09/04 12:51 PÆgina 597

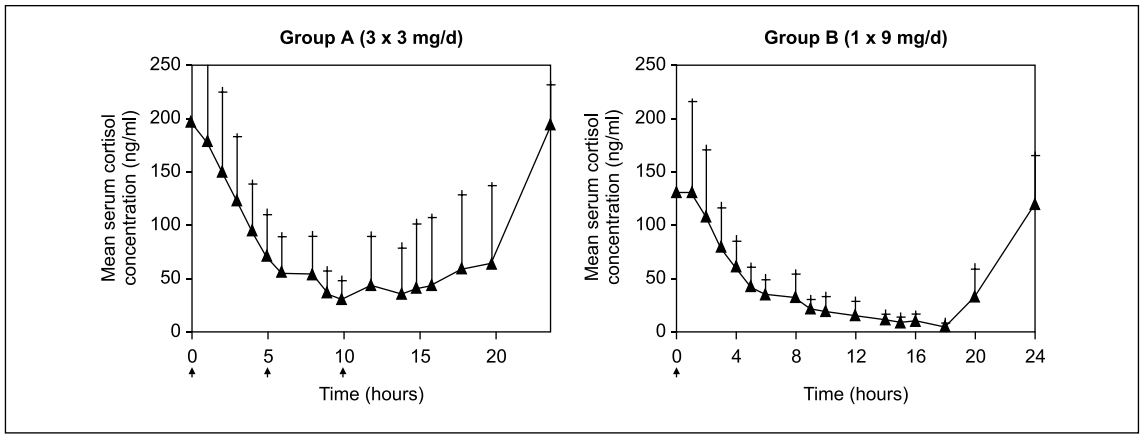

Fig. 4. Mean (± SD) serum cortisol concentrations after daily administration of 3 x 3 mg (group A) and 1 x 9 mg (group B) budesonide in patients with ulcerative colitis on day 5 of treatment.

Table VI: Rates of response and improvement after treatment with 3 x 3 mg (group A) or 1 x 9 mg (group B) budesonide daily on days 28 and 56.

	Number of patients			
	Response n (%)		Improvement n (%)	
ITT analysis (n = 15)	Day 28	Day 56	Day 28	Day 56
Group A: 3 x 3 mg/d budesonide (n = 8)	0	3 (38%)	0	3 (38%)
Group B: 1 x 9 mg/d budesonide (n = 7)	4 (57%)	4 (57%)	4 (57%)	5 (71%)
p-value*	0.026**	0.619	0.026**	0.315

* Fisher's exact test, two-sided.
**Statistically significant.

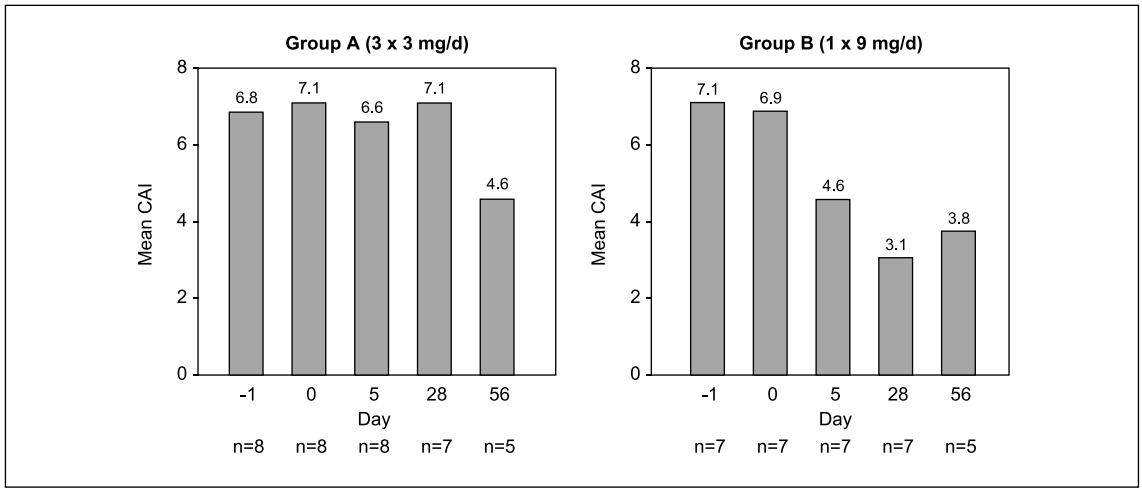

Figure 5. Mean clinical activity index (CAI) after the daily administration of 3 x 3 mg (group A) or 1 x 9 mg (group B) budesonide in patients with ulcerative colitis over 56 days.

Dr. Falk Pharma Ex. 1011
IPR Petition - USP 11,260,061

Kolkman-Pena.qxd 21/09/04 12:51 PÆgina 598

Table VII: Number of patients with at least one adverse event (AE) or potential adverse drug reaction (ADR).

	Number of patients ≥ 1	
Safety analysis (n = 15)	AE	ADR
Group A: 3 x 3 mg/d budesonide (n = 8)	2 (25%)	1 (13%)
Group B: 1 x 9 mg/d budesonide (n = 7)	3 (43%)	2 (29%)
Total	5 (33%)	3 (20%)

(range 0–100%, $p = 0.03$) in the 3 x 3 mg group and from median 100% (range 71–100%) to median 14% (range 0–100%, $p = 0.03$) in the 1 x 9 mg-group. The EI improved from 8.8 ± 2.1 to 3.8 ± 4.7 ($p = 0.02$) in the 3 x 3 mg group and from 9.7 ± 2.1 to 2.0 ± 2.9 ($p < 0.001$) in the 1 x 9 mg group. No changes in blood sedimentation rate or CRP concentrations were found during the study in either group. No decrease in serum aldosterone (measured at week 1, 4 and 8) was found. Mild adverse effects were reported in 5 subjects, gastrointestinal distress in 3, and nervousness and ankle edema in 1 (Table VII).

Discussion

This clinical study showed differences in the pharmacokinetics, pharmacodynamics and probably the clinical efficacy of oral budesonide with two different dosing regimens. It shows that the pH-modified release formulation of budesonide 3 mg capsules induced a treatment effect in about two-thirds of patients and gave rise to significant budesonide levels, especially in the 9 mg once-daily dosage regimen. It was surprising that this multicenter study recruited such a small number of patients and indicates the difficulty in performing pharmacokinetic and pharmacodynamic studies in clinical practice. Another limitation is the common observation that patients who presented with ulcerative colitis to the referral hospitals had already started treatment with other corticosteroids and could not be included in the study. This also contributed to the lack of standardization of the mesalazine used by patients although the dosage did not influence the assignment of patients to a particular group.

The once-daily dosage regimen had higher peak levels and slower absorption rates compared to the thrice-daily regimen, which confirms studies in healthy volunteers (19). On the other hand, the higher oral bioavailability and larger AUC with the once-daily regimen compared to the thrice-daily regimen contrasts findings in studies in healthy volunteers (19). Whether these differences relate to the seemingly better clinical response with the once-daily regimen remains uncertain at this point. Most other studies on the effects of topical glucocorticoids indicated that the effects of thrice-daily administration were superior to the same dose when administered once daily (19, 20). Clearly, additional comparative studies could elaborate whether these differences might be due in part to the slightly different patient population (differences in ethnic composition) or whether a saturable first-pass effect is responsible for the increased bioavailability of the 1 x 9 mg dosing regimen in the patient population. Lundin and *et al.* (18) reported an oral bioavailability of about 10% for a 1 x 9 mg dose in adults with active Crohn's disease. Edsbäcker and *et al.* (21) reported an oral bioavailaibility of 9–12% in healthy volunteers. Results for budesonide using pH-modified release capsules in healthy volunteers revealed similar AUC profiles with an oral bioavailability of about 10%. A nonlinearity between the pharmacokinetics of a 3 x 3 and 1 x 9 mg profile was, however, not observed in these studies (20). Differences in the intrinsic clearance of healthy volunteers and the patient population might be responsible for this observation. The results for budesonide mean pharmacokinetic parameters are also in reasonable agreement with an early publication that calculated budesonide kinetics after intravenous administration in healthy volunteers (22).

The mucosal levels of budesonide in the descending colon, sigmoid and rectum provide evidence that the budesonide from currently used budesonide capsule is still available at this level. The presence in baseline mucosal "budesonide levels" before the drug was even started indicates that some cross-reactivity of the budesonide assay with mucosal compounds was present. Still, the clear increase after 56 days treatment seems proof for the local availabilitiy and effect of the current formulation.

A difference in pharmacodynamic response between both drug regimens was noted with more pronounced cortisol suppression in the 9 mg once-daily regimen, although the effects on the circadian variation of lymphocyte and neutrophil counts did not differ. Because of the lack of actual placebo data, it was decided to neglect the small circadian

Dr. Falk Pharma Ex. 1011
IPR Petition - USP 11,260,061

Kolkman-Pena.qxd 21/09/04 12:51 PÆgina 599

rhythm and to only make valid conclusions about the direct comparison between the two dosing regimens. Previous studies for other glucocorticoids have shown that this approach is feasible because of relatively small variation over the day (23, 24). Patients' lymphocyte suppression in this study was in the range of 11.9 to 16.8% while the increase in neutrophils was in the range of 23.1 to 28.9%. When the cumulative lymphocyte and neutrophil estimates (AUC values) were compared for groups A and B, no significant differences between the two groups were observed. This is probably due to the overall relatively small effects observed at the indicated drug levels, and the variability of the data is distinct. Previous studies also indicated that lymphocytes and neutrophils are less sensitive markers of systemic effects than cortisol suppression (25).

Significant differences were found between the cortisol levels in groups A and B, indicating more pronounced cortisol suppression after the 1 x 9 mg treatment. The assessment of the cortisol data was also hampered by the fact that no full baseline profiles were available and that the design of the study was a randomized, parallel study design. Therefore, it can not be clearly concluded that the significant differences in the cortisol AUC_{0-24} between the 3 x 3 mg and 1 x 9 mg regimens are solely due to the dosing regimen, but may be also related to differences in the study populations. However, the observed differences in the systemic availability of budesonide between the two dosing regimens support the higher systemic cortisol suppression for the 1 x 9 mg dosing regimen.

For the first time, an efficacy of oral budesonide could be demonstrated in ulcerative colitis patients with inflammation limited to the distal parts of the colon. Obviously, the therapeutic effect is dependent on the dosage regimen. After 4 weeks of treatment, remarkably better results were found in the 1 x 9 mg regimen than in the 3 x 3 mg regimen: 57% *vs.* 0% of patients were in remission or showed clinical improvement. This difference was smaller after 8 weeks of treatment, when 57% (group B) *vs.* 38% (group A) were in remission. The onset of response indicated that the 1 x 9 mg treatment was not only more effective than the 3 x 3 mg treatment, but was also much faster in inducing remission or clinical improvement. It might be speculated that the higher efficacy of the 1 x 9 mg treatment might be related to the higher oral bioavailability of this dosing regimen. This is in agreement with the observation that the 1 x 9 mg treatment showed higher cortisol suppression than the 3 x 3 mg treatment. If this is the case, one might argue that at least a portion of the clinical effect is due to systemic action of budesonide.

The efficacy of oral budesonide demonstrated in this study is in agreement with the results of two earlier trials in ulcerative colitis. A controlled colonic release budesonide formulation in a daily dose of 10 mg in patients with mild to moderately active ulcerative colitis led to a significant improvement of the EI[10]. Twenty-one out of 34 patients (62%) under budesonide therapy improved endoscopically in this trial as compared to 4 out of 8 (50%) in the 3 x 3 mg group and 7 out of 7 (100%) in the 1 x 9 mg group in the study here presented. Another study on oral budesonide in ulcerative colitis investigated the efficacy and safety of the pH-modified release 3 mg capsules in steroid-dependent patients. A daily dose of 9 mg budesonide was well tolerated, significantly improving clinical symptoms and permitting the reduction of conventional, systemically acting steroids (12).

The pharmacokinetic investigations of the present study demonstrated therapeutically adequate mucosal budesonide concentrations in the inflamed regions of the sigmoid or rectum. It can be concluded that the active drug from both dosage regimens of budesonide 3 mg capsules is effective in the whole colon, reaching even distant distal gut regions. These results may explain the effectiveness of budesonide 3 mg capsules in the treatment of active ulcerative colitis and in Crohn's disease with inflammation not only in the ileocoecal gut region as previously demonstrated by Bar-Meir *et al.* (6) and Caesar *et al.* (26). In conclusion, this study provides the groundwork for potentially interesting validation that the topically acting oral budesonide is effective in distal active ulcerative colitis and has only a modest influence on systemic parameters. Previous studies have confirmed its efficacy in Crohn's disease and its good tolerability profile without serious drug-related adverse events as compared to the conventional, systemically acting steroids (6, 8). Thus, this topically acting, pH-modified release formulation of budesonide could be a treatment option in active ulcerative colitis but a larger clinical trial is mandatory.

Acknowledgements

The authors would like to thank the active participation of the gastroenterologists, F.G. Nelis from Isala Hospital (Sophia), Zwolle, and P. Viergever from the Gemini Hospital, Den Helder, the Netherlands.

Dr. Falk Pharma Ex. 1011
IPR Petition - USP 11,260,061

Kolkman-Pena.qxd 21/09/04 12:51 PÆgina 600

References

1. Campieri, M. *New steroids and new salicylates in inflammatory bowel disease: A critical appraisal.* Gut 2002, 50 (Suppl. III): iii43-iii46.
2. Kane, S.V., Schoenfeld, P., Sandborn, W.J., Tremain, W., Hofer, T., Feagn, B.G. *Systemic review: The effectiveness of budesonide therapy for Crohn's disease.* Aliment Pharmacol Ther 2002, 16: 1509-17.
3. Sandborn, W.J., Feagan, B.G. *Review article: mild to moderate Crohn's disease – defining the basis for a new treatment algorithm.* Aliment Pharmacol Ther 2003; 18: 263-77.
4. Andus, T., Gross, V., Caesar, I. et al. and the German/Austrian Budesonide Study Group. *Replacement of conventional glucocorticoids by oral pH-modified release budesonide in active and inactive Crohn's disease. Results of an open, prospective, multicenter trial.* Dig Dis Sci 2003, 48: 373-8.
5. Rutgeerts, P., Löfberg, R., Malchow, H. et al. *Comparison of budesonide with prednisolone for active Crohn's disease.* N Engl J Med 1994, 331: 842-5.
6. Bar-Meir, S., Chowers, Y., Lavy, A. et al. and the Israeli Budesonide Study Group. *Budesonide versus prednisone in the treatment of active Crohn's disease.* Gastroenterology 1998, 115: 835-40.
7. Caesar, I., Gross, V., Roth, M. et al. and members of the German Budesonide Study Group. *Treatment of active Crohn's ileocolitis with oral pH-modified budesonide. Germany Budesonide Study Group.* Z Gastroenterol 1995, 33: 247-50.
8. Gross, V., Caesar, I., Andus, T. et al. and the German/Austrian Budesonide Study Group. *Dose finding study with oral budesonide in patients with active Crohn's ileocolitis.* Gastroenterology 1997, 112: A986.
9. Greenberg, G.R., Feagan, B.G., Martin, F. et al. and the Canadian Inflammatory Bowel Disease Study Group. *Oral budesonide for active Crohn's disease.* N Engl J Med 1994, 331: 836-41.
10. Löfberg, R., Danielsson, Å., Nilsson, A. et al. *Oral budesonide versus prednisolone in patients with active extensive and left-sided ulcerative colitis.* Gastroenterology 1996, 110: 1713-8.
11. Deusch, K., Mauthe, B., Spießl, G., Classen, M. *Budesonide in the treatment of steroid dependent inflammatory bowel disease.* Falk Symposium No. 73. IV. Internationales Symposium über chronisch entzündliche Darmerkrankungen (Sep 6-8, Strassburg) 1993, Abstr. 29.
12. Keller, R., Stoll, R., Foerster, E.C., Gutsche N., Domschke, W. *Oral budesonide therapy for steroid-dependent ulcerative colitis: A pilot trial.* Aliment Pharmacol Ther 1997, 11: 1047-52.
13. Köhne, G. *Budesonide in the treatment of steroid dependent ulcerative pancolitis.* The World Congresses of Gastroenterology (Oct 2-7, Los Angeles) 1993, Abstr. II, 178P.
14. Hüppe, D., Schenck, B., Tromm, A., Bürk, G. *Budesonide treatment in a 14-year old girl with chronic active ulcerative colitis.* Falk Symposium No. 73. IV. Internationales Symposium über entzündliche Darmerkrankungen (Sep 6-8, Strassburg) 1993, Abstr. 51.
15. Möllmann, H.W., Hochhaus, G., Tromm, A. et al. *Pharmacokinetics and pharmacodynamics of budesonide-pH-modified release capsules.* In: Möllmann, H.W., May, B. (Eds.). Glucocorticoid Therapy in Chronic Inflammatory Bowel Disease – from Basic Principles to Rationale Therapy. Kluwer Academic Publishers, Dordrecht/Boston/London 1996, 107-20.
16. Rachmilewitz, D. (on behalf of an international study group). *Coated mesalazine (5-aminosalicylic acid) vs. sulphasalazine in the treatment of active ulcerative colitis: A randomised trial.* Br Med J 1989, 298: 82-6.
17. Dimova, H., Wang, Y., Pommery, S., Möllmann, H., Hochhaus, G. *SPE/RIA vs LC/MS for measurement of low levels of budesonide in plasma. Solid-phase extraction/radioimmuno-assay. Liquid chromatography/mas spectrometry.* Biomed Chromatogr 2003, 17: 14-20.
18. Lundin, P.D.P., Edsbäcker, S., Bergstrand, M. et al. *Pharmacokinetics of budesonide controlled ileal release capsules in children and adults with active Crohn's disease.* Aliment Pharmacol Ther 2003, 17: 85-92.
19. Hochhaus, G., Derendorf, H. *Dose optimization based on pharmacokinetic/pharmacodynamic modeling.* In: Derendorf, H., Hochhaus, G. (Eds.). Handbook of pharmacokinetic/pharmaco-dynamic correlations. CRC Press, Boca Raton/London/Tokyo 1995, 79-120.
20. Möllmann, H.W., Hochhaus, G., Tromm, A. et al. *Pharmacokinetics and pharmacodynamics of budesonide-pH modified release capsules.* Kongressband, IV International Symposium of Inflammatory Bowel Diseases. In: May, B., Möllmann, H. (Eds.). Kluwer, Academic Publishers, Dordrecht/Boston/London 1996, 107-20.

Dr. Falk Pharma Ex. 1011
IPR Petition - USP 11,260,061

Kolkman-Pena.qxd 21/09/04 12:51 PÆgina 601

21. Edsbacker, S., Bengtsson, B., Larsson, P. et al. *A pharmacoscintigraphic evaluation of oral budesonide given as controlled-release (Entocort) capsules.* Aliment Pharmacol Ther 2003, 17: 525-36.
22. Ryrfeldt, Å., Anderson, P., Edsbäcker, S., Tönnesson, M., Davies, D., Pauwels, R. *Pharmacokinetics and metabolism of budesonide, a selective glucocorticoid.* Eur J Respir Dis 1982, 63 (Suppl. 122): 86-95.
23. Möllmann, H., Hochhaus, G., Rohatagi, S., Barth, J., Derendorf, H. *Pharmacokinetic/pharmacodynamic evaluation of deflazacort in comparison to methylprednisolone and prednisolone.* Pharm Res 1995; 12: 1096-100.
24. Derendorf, H., Hochhaus, G., Rohatagi, S. et al. *Pharmacokinetics of triamcinolone acetonide after intravenous, oral and inhaled administration.* J Clin Pharmacol 1995, 35: 302-5.
25. Rohatagi, S., Hochhaus, G., Möllmann, H. et al. *Pharmacokinetic and pharmacodynamic evaluation of triamcinolone acetonide after intravenous, oral, and inhaled administration.* J Clin Pharmacol 1995, 35: 1187-93.
26. Caesar, I., Gross, V., Roth, M. et al. and the members of the German Budesonide Study Group. *Treatment of active and postactive ileal and colonic Crohn's disease with oral pH-modified-release budesonide.* Hepato-Gastroenterology 1997, 44: 445-51.

Dr. Falk Pharma Ex. 1011
IPR Petition - USP 11,260,061