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Pharmacokinetics and Pharmacodynamic Action of Budesonide after Buccal Administration in Healthy Subjects and Patients with Oral Chronic Graft-versus-Host Disease

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Buccal administration of budesonide (mouthwash) may be effective as a topical add-on therapy in patients with oral chronic graft-versus-host disease (cGVHD). Safety of approved oral budesonide is based on high intestinal and hepatic extraction by cytochrome P450 3A (CYP3A) enzymes. The purpose of this study was to evaluate the presystemic extraction and pharmacodynamic action of buccal budesonide. Oral budesonide (3 mg) was taken as reference to which various single and multiple dose regimens of buccal budesonide were compared. Budesonide and the 2 main CYP3A-dependent metabolites (6β-hydroxybudesonide, 16α-hydroxyprednisolone) were analyzed in blood and urine along with the drug's effect on endogenous cortisol in 12 healthy subjects and 7 patients with oral cGVHD. We assessed CYP3A-dependent metabolites in both healthy subjects and patients after buccal budesonide. Whereas systemic exposure to budesonide was markedly lower in healthy subjects after the mouthwash compared to oral dosing (mean relative bioavailability 18%–36%), the systemic concentrations thereafter in patients were as high as those after the identical dose of oral budesonide. Reduced buccal CYP3A activity (lower inactivation of budesonide) in patients contributed to this remarkable difference. Endogenous cortisol was suppressed in some patients during 1 week of continuous treatment with buccal budesonide (3 × 3 mg per day). We are the first to report the biotransformation of budesonide via CYP3A enzymes after buccal drug administration. Only 2% of a buccal dose of budesonide achieves systemic circulation in healthy individuals; that fraction is 10% in patients with oral cGVHD, probably because of alterations in drug uptake and metabolization.

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INTRODUCTION

Between 20% and 50% of patients surviving more than 6 months after allogenic hematopoietic stem cell transplantation (HSCT) for hematologic malignancies will develop some degree of chronic graft-versus-host disease (cGVHD) [1]. GVHD is the result of allogenic T cells (either transferred with the donor's stem cell inoculum or developing from it) reacting with antigenic targets on host cells [2]. Its clinical manifestations determine whether the clinical syndrome of GVHD is classified as acute (aGVHD) or cGVHD [3]. Oral cGVHD resembles an autoimmune disorder with skin, liver, oral, and ocular lesions being the predominant manifestations [4]. Systemic prednisone, together with a calcineurin inhibitor such as cyclosporine (CsA) or tacrolimus, is the standard treatment of cGVHD at present [5]. However, oral cGVHD is often refractory to conventional therapy [6]. To date, there is no specific, undisputed treatment of oral cGVHD that convinces [7,8].

Budesonide is a relatively new synthetic glucocorticoid demonstrating a high ratio of local to systemic anti-inflammatory activity [9]. Two recent clinical investigations reported that the buccal administration of budesonide may be effective as topical add-on therapy for oral cGVHD [10,11]. Oral budesonide has been approved for the treatment of mild to moderate exacerbations of Crohn's disease for many years [12].

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The low risk of adverse drug reactions to oral budesonide is attributed to its low absolute bioavailability because of extensive presystemic biotransformation via cytochrome P450 3A (CYP3A) enzymes into the 2 major and pharmacologically inactive metabolites 6β-hydroxybudesonide and 16α-hydroxyprednisolone [13]. CYP3A enzymes are the most abundant and predominant drug-metabolizing enzymes in humans [14]. However, unlike the gut and liver, the oral mucosa has not been thoroughly characterized in terms of its CYP3A expression [15,16]. There are no data on buccal expression of CYP3A in patients with oral cGVHD. Therefore, clinical studies on the disposition of buccal budesonide in patients with oral cGVHD, including characterization of the relevant metabolic pathways, are necessary. A source of concern regarding the topical administration of budesonide in oral cGVHD is that it could lead to significant systemic levels of the steroid. As systemic exposure to topical steroids and topical tacrolimus has been reported in several papers [17-19], it is important to know whether there is a significant degree of direct buccal absorption of budesonide.

The aim of our trial was thus to determine the pharmacokinetic profile and pharmacodynamic action of buccal budesonide following single-dose and steady-state dosing. A thorough analysis of metabolite kinetics (formation of 6β-hydroxybudesonide and 16α-hydroxyprednisolone via CYP3A) is provided. To determine the safe dosing of buccal budesonide, we aimed to examine its effects on endogenous cortisol production.

MATERIALS AND METHODS

Subjects

Enrolled in this study were 12 healthy subjects (6 females, 6 males; 43.7 ± 7.1 years, 71.5 ± 10.3 kg) and 7 patients (1 female, 6 males; 44.4 ± 14.0 years, 66.6 ± 9.1 kg) presenting with at least 1 of 5 diagnostic clinical signs of oral cGVHD (lichen-type features, hyperkeratotic plaques, ulcers, pseudomembranes, or decreased oral range of motion in patients with sclerotic skin features of GVHD [3,20]). Oral cGVHD had to have occured beyond 100 days after HSCT Only patients who presented a lack of complete response following conventional first-line treatment (systemic glucocorticoids and/or calcineurin inhibitors), that is, they still showed signs and symptoms of oral GVHD, were included. One main exclusion criterion was oral mucositis resulting from chemotherapy or radiotherapy. Any change in the dosage regimen of concomitant glucocorticoids or calcineurin inhibitors was not allowed within 3 weeks before and during the trial; repeated use of CYP3A inducers or inhibitors was not allowed; an exception was made regarding a stable regimen of azole antifungals [21]. Healthy subjects were excluded if they had taken any medications within 2 weeks before or during the study. Grapefruit consumption in the week prior to the first study day or during the trial precluded participation in all subjects.

Study Design

First, healthy subjects received aqueous solutions of budesonide tablets (1 effervescent buccal tablet containing 3 mg budesonide) in a fixed treatment order comprising 6 different treatments: (1) as a reference to calculate relative bioavailability of the oral intake (immediate swallowing) of 10 mL aqueous solution containing 3 mg budesonide [R]; thereafter (2) a single dose of 10 mL aqueous solution containing 3 mg budesonide as a 10-minute mouthwash [SD1]; (3) a single dose of 10 mL aqueous solution containing 3 mg budesonide as a 5-minute mouthwash [SD2]; (4) a single dose of 10 mL aqueous solution containing 6 mg budesonide as a 10-minute mouthwash [SD3]; (5) a single dose of 10 mL aqueous solution containing 9 mg budesonide as a 10-minute mouthwash [SD4]; and finally, (6) multiple doses of 10 mL aqueous solution containing 3 mg budesonide for 7 days (3 times per day, making a total daily dose of 9 mg) as a 10-minute mouthwash, with the last mouthwash on the morning of day 7 [MD1]. Second, patients with oral cGVHD received aqueous solutions of budesonide via only 3 of the above treatments: (1) for reference purposes, the oral intake of 10 mL aqueous solution containing 3 mg budesonide [R]; thereafter (2) a single dose of 10 mL aqueous solution containing 3 mg budesonide as a 10-minute mouthwash [SD1]; and finally, (3) multiple doses of 10ml aqueous solution containing 3 mg budesonide for 7 days (3 times per day, making a total daily dose of 9 mg) as a 10-minute mouthwash with the final mouthwash on the morning of day 7 [MD1]. Correct oral intake and correct buccal drug administration (eg, keeping the solution in the mouth for 10 minutes before expectorating) was supervised by an investigator. Single-dose administrations were separated by a washout of at least 3 days, the multiple-dose period followed the last single-dose administration without a washout. All drug administrations for pharmacokinetic and dynamic profiling were performed after an overnight fast, and subjects continued to fast for another 2 hours; fluid was not allowed during the first hour after the mouthwash.

Complete organ staging of cGVHD was carried out in the patients within 2 weeks prior to the first study day; skin, mouth, eyes, gastrointestinal tract, liver, lungs, joints, and fascia and genital tract were rated separately with each score ranging from 0 to 3 [3]. Systematic clinical assessment of the oral mucosa

was done on the first and last study days [22]. The 9 areas examined for the Oral Mucosa Rating Scale (OMRS) were: upper and lower lips, upper and lower labial mucosa, right and left buccal mucosa, dorsal and lateroventral tongue, soft palate. Erythema, lichenoid hyperkeratosis, and pseudomembranes/ulceration were assigned scores ranging from 0 to 3 resulting in a maximum total score of 81. To identify any oral mucoceles, we examined the soft palate, lower labial, and buccal mucosa in a standardized procedure.

Single-dose and steady-state (last day of treatment MD1) pharmacokinetic and dynamic profiling was done by measuring budesonide, CYP3A-dependent metabolites (6β-hydroxybudesonide, 16α-hydroxyprednisolone) in plasma and urine, and cortisol in serum and urine over a period of 12 hours after drug administration. To be precise, blood samples for budesonide and metabolites were taken just before and 10, 20, 30, 40, 60, 80, and 100 minutes, as well as 2, 3, 4, 5, 6, 8, and 12 hours after drug administration; blood samples for cortisol were taken just before and 2, 4, 6, 8, and 12 hours after drug administration. Urine was collected at 1 interval (0-12 hours). Plasma, serum, and urine were stored at −20°C (<3 months) until analysis.

Analytical Methods

Concentrations of budesonide, 6β-hydroxybudesonide, and 16α-hydroxyprednisolone in plasma were determined by validated liquid chromatography tandem mass spectrometry as described previously [23]. The lower limits of quantification in plasma (urine) were 0.1 ng/mL (0.5 ng/mL) for budesonide and 6β-hydroxybudesonide, and 0.5 ng/mL (2 ng/mL) for 16α-hydroxyprednisolone. Between-day and within-day coefficients of variation of quality controls were under 15%. Cortisol in serum and urine was determined using a solid-phase, competitive chemiluminescent enzyme immunoassay (IMMULITE 2000, Diagnostic Products, Los Angeles, CA). Analytical sensitivity of the test was 0.2 μg/dL.

Pharmacokinetic and Pharmacodynamic Analyses

Peak plasma concentration (C_{max}), and time of C_{max} (t_{max}) were obtained directly from the plasma concentration-time curves. The area under the plasma concentration-time curve (AUC) represents the extent of systemic drug exposure. AUC and terminal elimination half-life ($t_{1/2} = \ln[2]/\lambda$) were calculated using standard noncompartmental analysis (WinNonlin v. 4.1, Pharsight Corporation, Mountain View, CA). The elimination-rate constant (λ) was determined by linear regression analysis of the terminal log-linear phase of the plasma concentration-time curve. Relative bioavailability (F_{rel}) of budesonide after buccal administration as compared to oral intake was calculated by ($AUC_{0\text{-}tlast,buccal}$ * oral dose)/($AUC_{0\text{-}tlast,oral}$ * buccal dose). Rate of accumulation during steady-state dosing was obtained by the following ratio [24]: $R_{ac} = AUC_{ss,0\text{-}8h}/AUC_{0\text{-}8h,SD1}$. A linearity factor of pharmacokinetics after repeated drug administration was calculated as the ratio of $AUC_{ss,0\text{-}8h}$ to $AUC_{0\text{-}\infty,SD1}$. Molar ratios of metabolite formation ($AUC_{Met}/AUC_{Budesonide}$, where Met is the metabolite), such as $AUC_{0\text{-}tlast}$ of 6β-hydroxybudesonide to $AUC_{0\text{-}tlast}$ of budesonide, were used as indices of CYP3A metabolic activity. Urinary recoveries of the analytes were based on the cumulative amount of the analyte excreted during the 12-hour collection period ($Ae_{0\text{-}12h}$), and expressed as a percentage of the budesonide dose administered after correction for molecular weight. Effects of budesonide on endogenous cortisol production were evaluated in each individual by (1) measuring morning cortisol serum levels (8 A.M.), by (2) calculating AUC of cortisol in plasma over 12 hours, and by (3) measuring the cumulative amount of cortisol excreted into urine over 12 hours.

Statistical Analysis

The number of subjects was chosen in accordance with accepted guidelines for investigating bioavailability [25]. All parameters are given as mean ± SD or median with range in parentheses. Repeated-measures ANOVA was performed to assess differences between the treatments in each group of subjects. Differences in pharmacokinetic parameters between healthy subjects and patients with oral cGVHD were tested for significance using the Mann-Whitney test. $P < .05$ was regarded as statistically significant. Statistical comparison was carried out using GraphPad Instat software (GraphPad Software, Inc., San Diego, CA).

Ethical Considerations

The study protocol was approved by the institutional ethics committees of the participating centers. The trial was conducted from February 2006 until October 2007 in accordance with the ethical guidelines of the Declaration of Helsinki, and International Conference on Harmonization guidelines for Good Clinical Practice. All participants gave written informed consent. Budesonide was designated as an orphan medicinal product in the European Union intended for the treatment of resistant oral cGVHD (EU/3/06/413).

RESULTS

All participants completed the study according to the protocol with excellent compliance. New onset of oral candidiasis was reported in 2 patients. There were no significant changes in how the oral mucosa was rated during the trial (17.4 ± 4.2 versus 15.4 ± 10.0, OMRS).

Table 1. Absorption of Budesonide in 12 Healthy Subjects following Five Different Single Dose Administrations

	C_{max} (ng/mL)	t_{max} (hours)	$AUC_{0\text{-tlast}}$ (h*ng/mL)	F_{rel} (%)
R, oral intake (3 mg)	1.23 ± 0.52***	1.2 (0.3-1.7)*	2.67 ± 1.09$^{\#\#\#\#,\#\#}$	(comparator)
SD1, buccal (3 mg, 10-minute mouthwash)	0.18 ± 0.10	1.7 (1.3-4.0)	0.35 ± 0.35	18 ± 22
SD2, buccal (3 mg, 5-minute mouthwash)	0.27 ± 0.12	1.7 (0.7-2.0)	0.71 ± 0.57	36 ± 32$^{\#}$
SD3, buccal (6 mg, 10-minute mouthwash)	0.41 ± 0.22	1.7 (1.0-2.0)	1.30 ± 0.83$^{\$\$}$	33 ± 30
SD4, buccal (9 mg, 10-minute mouthwash)	0.66 ± 0.36**,*	1.7 (1.0-2.0)	2.06 ± 1.20$^{\$\$\$,\$\$}$	32 ± 24

C_{max} indicates maximum plasma concentration; t_{max}, time to maximum plasma concentration; $AUC_{0\text{-tlast}}$, area under the plasma concentration-time curve until last observed concentration; F_{rel}, relative bioavailability comparing buccal with oral drug administration.
***P < .001 versus SD1-4; **P < .01 versus SD1; *P < .05 versus SD2; $^{\#}P$ < .05 versus SD1; $^{\#\#}P$ < .01 versus SD3; $^{\#\#\#\#}P$ < .001 versus SD1-2; $^{\$\$}P$ < .01 versus SD2; $^{\$\$\$}P$ < .001 versus SD1; repeated-measures ANOVA.
Mean ± SD or median with range in parentheses.

Pharmacokinetics

Healthy subjects

Pharmacokinetic parameters of budesonide following the 5 different single dose administrations are given in Table 1. Corresponding plasma concentration-time curves are shown in Figure 1. Systemic exposure to budesonide was markedly lower after buccal drug administration than after oral dosing (see mean F_{rel} of budesonide, 18%-36%). Comparison of peak plasma concentrations (C_{max}) and AUC, which are the major parameters characterizing drug absorption, revealed higher systemic drug exposure following the administration of higher doses of buccal budesonide. Formation of CYP3A-dependent metabolites, in particular that of 6β-hydroxybudesonide, was higher after the oral intake of budesonide (4.7 ± 1.7, $AUC_{6\beta\text{-hydroxybudesonide}}/AUC_{Budesonide}$) than after buccal drug administration ($P < 0.001$ versus each mouthwash), with no significant differences among the 4 buccal administrations (1.0 ± 1.0, SD1; 0.9 ± 0.4, SD2; 1.7 ± 0.7, SD3, 1.9 ± 0.7, SD4). Repeated doses of buccal budesonide (3 × 3 mg per day) over 1 week resulted in minor systemic drug accumulation (1.6 ± 0.6, budesonide; 1.8 ± 0.6, 6β-hydroxybudesonide; 1.4 ± 0.6, 16α-hydroxyprednisolone; R_{ac}).

Patients with Oral cGVHD

The patients' demographic and clinical features are listed in Table 2. The comparison of oral and buccal drug disposition in healthy subjects and patients is illustrated in Figure 2. Pharmacokinetic parameters following the first mouthwash are listed in Table 3 (comparing healthy and ill states). Statistical analysis revealed significant differences in C_{max} and AUC of budesonide between healthy subjects and patients. Surprisingly, the patients' systemic exposure to budesonide after buccal administration resembled that after oral dosing (Table 3, mean F_{rel} of budesonide mouthwash 100%); this was not the case in healthy subjects. Formation of CYP3A-dependent metabolites of budesonide was observed in healthy subjects and patients. Ratios of metabolite formation reflecting CYP3A activity are presented in Figure 3. Formation of 16α-hydroxyprednisolone (but not that of 6β-hydroxybudesonide) was significantly impaired after buccal drug administration in patients with oral cGVHD in comparison to healthy subjects. As had been the case in healthy subjects, steady-state dosing resulted in minor systemic drug accumulation in patients (R_{ac}: 1.8 ± 0.9, budesonide; 2.6 ± 2.1, 6β-hydroxybudesonide; 2.3 ± 2.0, 16α-hydroxyprednisolone). The linearity factor based on AUC of budesonide was 1.3 ± 0.6 in both patients and healthy subjects.

Pharmacodynamic Action

Predose morning serum cortisol (normal 5-25 μg/dL) on the first study day was lower in patients (range: 4.95-8.08 μg/dL) than in healthy subjects (range: 10.92-14.01 μg/dL). This finding may result from comedications; 6 of 7 patients were on a stable dose of oral prednisone (see Table 2). Most relevant for evaluating the pharmacodynamics of buccal budesonide is the comparison of morning serum cortisol at baseline (R) with that after 1 week of continuous treatment (MD1). The patients' values were 7.18 ± 6.16 μg/dL for R and 4.95 ± 5.84 μg/dL for MD1, and 11.73 ± 4.06 μg/dL for R, and 14.01 ± 4.34 μg/dL for MD1 in healthy subjects, respectively (no significant changes). After 1 week of budesonide-rinsing (MD1) we observed suppressed cortisol profiles (AUC) in 4

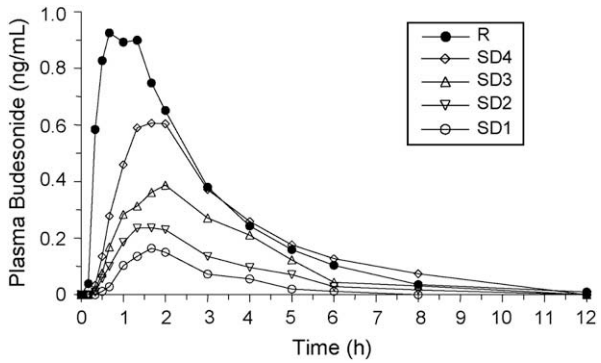

Figure 1. Plasma budesonide concentration-time curves in 12 healthy subjects following 5 different treatments with single doses of budesonide: R (oral intake); SD1, SD2, SD3, SD4 (mouthwash, for details see study design). Data are presented as mean.

Table 2. Demographic and Clinical Features of the Patient Population at Baseline

Patient	Age (Years)	Sex (m/f)	Height (cm)	Weight (kg)	Organ Scoring of cGVHD (0-3)* [3]	OMRS (0-81) [22]	Oral Mucoceles	Concomitant Prednisone (Oral Dose per Day, Duration)
1	53	M	181	64	mouth 2, eyes 2, GI tract 1, lungs 2	13	+++	4.5 mg, 121 days
2	48	F	156	64	skin 2, mouth 2, eyes 1, lungs 1	12	—	—
3	59	M	183	71	mouth 2, eyes 2, GI tract 2	24	—	12.5 mg, 35 days
4	32	M	175	50	skin 2, mouth 2, eyes 2, GI tract 1, joints and fascia 1	18	—	15 mg, 81 days
5	60	M	182	80	skin 1, mouth 1, eyes 3, liver 2, joints and fascia 1, genital tract 2	19	+	5 mg, 54 days
6	34	M	172	67	mouth 2, eyes 1 ,	16	—	10 mg,† 106 days
7	25	M	192	70	skin 1, mouth 2, GI tract 1, liver 1	20	+	20 mg, 74 days
Mean	44.4		177.3	66.6		17.4		9.6 mg, 79 days
SD	14.0		11.3	9.1		4.2		6.9 mg, 32 days

GVHD indicates graft-versus-host-disease; OMRS, oral mucosa rating scale; GI, gastrointestinal.
*Organ with score 0 (no symptoms/normal) not given.
†Fifteen milligrams during the initial 35 of 106 days.

of 7 patients. Morning cortisol levels at MD1 did not significantly correlate to dose or duration of concomitant oral prednisone (Spearman rank correlation). Likewise, urinary cortisol excretion was lower in patients with oral cGVHD than in healthy subjects. We observed a dose-response relationship with urinary amounts of cortisol decreasing with higher daily doses of buccal budesonide in healthy subjects. The effect of budesonide on endogenous cortisol in healthy subjects and patients is summarized in Table 4.

DISCUSSION

This is the first report on the systemic bioavailability of budesonide after buccal administration. Our study provides comprehensive data on the pharmacokinetics and pharmacodynamic action of buccal budesonide in healthy subjects and patients with oral cGVHD. Oral intake of budesonide was chosen as the reference standard to which we compared the buccal administrations of budesonide. Budesonide is regarded as a sensitive substrate of CYP3A enzymes [26]. Because budesonide is eliminated via degradation into inactive phase-I metabolites, its elimination clearance largely depends on the activity of these drug-metabolizing enzymes. In humans, approximately 60% of the total hepatic CYPs belong to the CYP3A subfamily [27]. CYP3A is the main CYP found in the small intestinal epithelia and livers of adult humans [28,29].

Physiologically speaking, the blood supply from the gastrointestinal tract passes through the gut and liver on its way to the heart and lungs. The entire dose of oral budesonide is thus subject to extensive presystemic extraction. According to the literature, oral budesonide has a very low *absolute* bioavailability (F_{abs}) of only 10% because of a high first-pass effect [30]. It is important to emphasize the distinction between absolute and relative bioavalability [31]. Absolute bioavailability of oral budesonide is the measure of systemic exposure of orally dosed budesonide relative to intravenously given budesonide. The *relative* bioavailability (F_{rel}) of buccal budesonide calculated in our trial describes the systemic exposure after budesonide mouthwash compared to an accepted standard (oral budesonide). It follows that only a very small fraction of buccally applied budesonide reaches systemic circulation in healthy subjects (about 2% of the active substance, calculated from 18% [F_{rel}] of 10% [F_{abs}]). Surprisingly, the buccal-dose fraction that reaches the systemic circulation is higher in

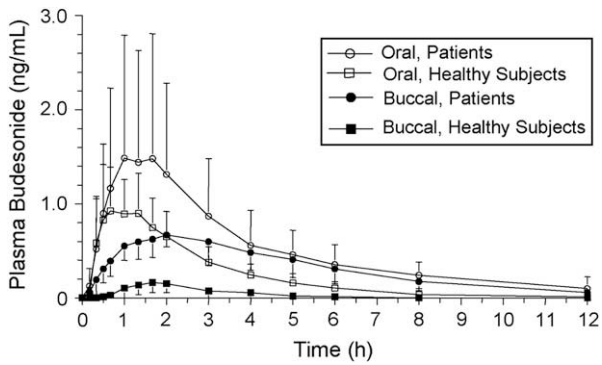

Figure 2. Plasma budesonide concentration-time curves in 12 healthy subjects (square) and 7 patients with oral cGVHD (circle) following a single dose of 3 mg budesonide taken orally (open) or administered buccally (closed). Data are presented as mean ± SD.

Table 3. Pharmacokinetic Parameters of Budesonide and 2 CYP3A-Dependent Metabolites in 12 Healthy Subjects and in 7 Patients with Oral cGVHD following Buccal Drug Administration (SD1)

	Healthy Subjects	Patients	Mann-Whitney Test
Budesonide			
C_{max} (ng/mL)	0.18 ± 0.10	0.77 ± 0.23	$P < .0001$
t_{max} (h)	1.7 (1.3-4.0)	2.0 (1.0-3.0)	n.s.
$AUC_{0-\infty}$ (h*ng/mL)	1.14 ± 0.39	4.37 ± 1.30	$P < .01$
$t_{1/2}$ (h)	2.8 (1.9-5.0)	3.0 (2.5-6.3)	n.s.
F_{rel} (%)	18 ± 22	100 ± 98	$P < .01$
Ae_{0-12h} (% of dose)	0 (0)	0 (0-0.03)	n.s.
6β-Hydroxybudesonide			
C_{max} (ng/mL)	0.15 ± 0.12	0.52 ± 0.28	$P < .01$
t_{max} (h)	1.7 (0.7-3.0)	1.8 (1.0-6.0)	n.s.
$AUC_{0-\infty}$ (h*ng/mL)	2.00 ± 1.05	4.41 ± 1.95	$P < .01$
Ae_{0-12h} (% of dose)	0.16 (0-0.56)	0.24 (0.12-0.53)	n.s.
16α-Hydroxyprednisolone			
C_{max} (ng/mL)	2.39 ± 1.27	5.11 ± 4.91	n.s.
t_{max} (h)	1.7 (1.0-2.0)	1.8 (1.0-6.0)	n.s.
$AUC_{0-\infty}$ (h*ng/mL)	8.38 ± 3.96	22.32 ± 11.33	$P < .01$
Ae_{0-12h} (% of dose)	1.21 (0-5.25)	1.21 (1.02-4.52)	n.s.

C_{max} indicates maximum plasma concentration; t_{max}, time to maximum plasma concentration; $AUC_{0-\infty}$, area under the plasma concentration-time curve extrapolated to infinity; $t_{1/2}$, terminal elimination half-life; Ae_{0-12h}, amount excreted into urine over 12 hours; n.s., not significant. Mean ± SD or median with range in parentheses.

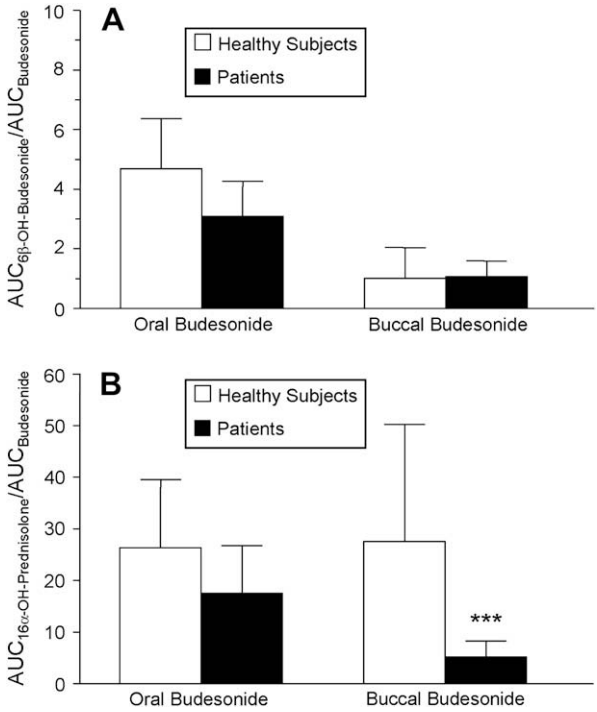

Figure 3. Ratios of CYP3A-dependent metabolite formation following a single oral dose of 3 mg budesonide by different routes of drug administration in 12 healthy subjects (white) and 7 patients with oral cGVHD (black). Data are presented for 6β-hydroxybudesonide (A) and 16α-hydroxyprednisolone (B) as mean and SD. $P < .001$ versus healthy subjects, Mann-Whitney test. ***$P < 0.001$.

patients, or about 10% (100% [F_{rel}] of 10% [F_{abs}]), but it does not exceed the fraction of the oral dose reaching systemic circulation. The blood supply from the oral cavity passes the oral mucosa as the barrier to drug absorption on its way to the heart and lungs; it does not pass through the gut and liver.

CYP3A enzymes were recently detected in the oral mucosa. Semiquantitative comparison of the DNA bands revealed that CYP3A5 expression is similar in human liver and oral buccal-tissue samples [15]. An RT-PCR analysis by other investigators revealed that the more common subtype CYP3A4 is also expressed together with CYP3A5 in human buccal tissue [16]. CYP3A enzymes have not yet been specifically localized in the buccal mucosa. Our clinical results concur with these preliminary in vitro findings. The parent compound and both CYP3A-dependent metabolites were detected in each subject in venous blood samples after the buccal administration of budesonide. As shown in our analysis of metabolite kinetics, oral cGVHD affects buccal CYP3A enzyme activity.

An additional reason behind the obvious difference in the disposition of budesonide between healthy subjects and patients with oral cGVHD could be altered drug uptake in the oral mucosa. In contrast to the gastrointestinal tract, much less is known about the type and capacity of drug-transport processes in buccal-epithelial cells [32]. Budesonide has been identified as a substrate of the drug-efflux pump P-glycoprotein that shares considerable substrate-specificity with CYP3A4 [33]. P-glycoprotein is located in the apical (luminal) membrane of enterocytes between the duodenum and colon [34]. P-glycoprotein limits drug absorption by pumping its substrates back into the gut lumen [35]. There are no data on the expression of P-gp in the oral cavity other than 2 pilot reports on the expression of P-gp in human gingiva and buccal epithelium [36,37]. We assume that alterations in drug transport in the oral cavity of patients with oral cGVHD result in increased absorption of buccal budesonide. Increased uptake of budesonide via impaired mucosa (as an underlying reason for higher plasma levels) is supported by the fact that the elimination half-life of buccal budesonide was not longer in patients than in healthy subjects. Further investigations addressing the role of drug-transporting proteins and the interplay with drug-metabolizing enzymes in the human oral cavity (including the analysis of oral tissue samples) are now warranted from the clinical-pharmacological perspective.

We cannot definitely exclude minor hepatic extraction of budesonide because of "second pass." This may account for some of the reported findings. The particular role of the hepatic manifestation of cGVHD for biotransformation of buccal budesonide could not be determined in this study because of the low number of patients (2 of 7 patients had liver manifestation). However, this particular aspect is a challenge for future investigations.

In our trial, pharmacodynamics focused on serum cortisol concentrations. This method is used to detect

Table 4. Effect of Budesonide on Endogenous Cortisol in 12 Healthy Subjects and 7 Patients with Oral c GVHD Comparing Oral Intake (R), Single-Dose Buccal Drug Administration (SD1), and Buccal Drug Administration after Thrice-Daily Dosing during 1 Week (MD1)

Evaluation of cortisol	R	SD1	MD1
C_{0h} (μg/dL)			
healthy subjects	11.73 ± 4.06	10.92 ± 3.39	14.01 ± 4.34*
patients	7.18 ± 6.16	8.08 ± 6.65	4.95 ± 5.84##
C_{12h} (μg/dL)			
healthy subjects	7.81 ± 8.50	4.92 ± 3.37	3.93 ± 1.84
patients	1.58 ± 1.33####	3.47 ± 2.47	2.20 ± 1.66
$AUC_{0\text{-}12h}$ (h*μg/dL)			
healthy subjects	73 ± 22	79 ± 27	80 ± 23
patients	34 ± 23##	45 ± 23#	35 ± 25##
$Ae_{0\text{-}12h}$ (μg)			
healthy subjects	95 ± 64	74 ± 58	44 ± 33*,***
patients	30 ± 33##	22 ± 31##	25 ± 37

C_{0h} indicates predose cortisol plasma concentration (8 A.M.); C_{12h}, cortisol plasma concentration at 12 hours (8 P.M.); $AUC_{0\text{-}12h}$, area under the cortisol plasma concentration-time curve during 12 hours; $Ae_{0\text{-}12h}$, amount of cortisol excreted into urine over 12 hours.
*P < .05 versus SD1, ***P < .001 versus R; repeated-measures ANOVA. #P < .05 versus healthy subjects, ##P < .01 versus healthy subjects ####P < .001 versus healthy subjects; Mann-Whitney test.
Data are given as mean ± SD.

adrenal suppression before the appearance of clinical symptoms. We observed signs of a possible effect on endogenous cortisol secretion during the brief add-on use of budesonide mouthwash in patients with oral cGVHD. This should be examined in more detail in a subsequent long-term study.

In conclusion, we are the first to report the metabolization of buccal budesonide via CYP3A enzymes in healthy subjects and patients with oral cGVHD. The remarkable difference in systemic exposure to buccal budesonide between healthy subjects and patients seems to be because of the increased drug transport and impaired CYP3A activity in the oral mucosa of patients with oral cGVHD. We noted no new signs of risk associated with the buccal administration of budesonide when considering the known safety profile of oral budesonide.

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