Budesonide use and misuse in sports: elimination profiles of budesonide and metabolites after intranasal, high-dose inhaled and oral administrations.

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Abstract

Budesonide (BUD) is a glucocorticoid (GC) widely used in therapeutics. In sports, the World Anti-doping Agency (WADA) controls the use of GCs, and WADA-accredited laboratories use a reporting level of 30 ng/mL for 6 β -hydroxy-budesonide (6 β OHBUD) to detect the systemic administration of BUD. In the present work, we examined the urinary excretion profile of 6 β OHBUD, BUD, and 16 α -hydroxy-prednisolone (16 α OHPRED) after intranasal (INT), inhaled (INH) (at high doses) and oral administrations in male and female volunteers.

BUD was administered to healthy volunteers using INT route (256 μ g/day for three days, n= 4 males and 4 females), INH route (800 μ g/day for three days, n= 4 males and 4 females and 1600 μ g/day for three days, n=4 males) or oral route (3 mg, n= 8 females). Urine samples were collected before and after administration at different time periods, and were analysed by liquid chromatography-tandem mass spectrometry.

6 β OHBUD and BUD concentrations were very low after INT treatment (0.0-7.1 and 0.0-8.1 ng/mL, respectively), whereas they were higher after INH treatments (0.0-35.4 and 0.0-48.3 ng/mL, respectively). For 16 α OHPRED, elevated concentrations were detected after INT and INH treatments (2.6-66.4 and 3.4-426.5 ng/mL, respectively). Concentrations obtained following oral administration were higher than after therapeutic administrations (2.8-80.6, 1.5-36.1 and 10.4-532.2 ng/mL for 6 β OHBUD, BUD and 16 α OHPRED, respectively). After all administrations, concentrations were higher in males than in females.

Results demonstrated that 6 β OHBUD is the best discriminatory marker and a reporting level of 40 ng/mL was found to be the best criterion to distinguish allowed from forbidden administrations of BUD.

1. Introduction

Budesonide (BUD) is a glucocorticoid (GC) commonly used in therapeutics by different administration routes such as inhalation (INH), in the treatment of asthma (1), intranasal (INT), in the treatment of rhinitis (1), and oral administrations, for the inflammatory bowel disease (2).

GCs are prohibited in sports because of evidences of positive effects on exercise performance and health risks associated to their use due to the inhibition of the hypothalamic-pituitary-adrenal axis (3-5). GCs are prohibited in competitions when administered by systemic routes (oral, intramuscular, intravenous or rectal routes), and they are allowed for therapeutic purposes using other administration routes considered of local action (INT and INH administrations, among others).

World Anti-Doping Agency (WADA)-accredited laboratories detect the systemic administration of BUD by targeting its metabolite, 6 β -hydroxy-budesonide (6 β OHBUD), with a reporting level of 30 ng/mL (6). This criterion was established based on studies performed in our group (7, 8). First, the urinary metabolic profile of BUD was evaluated after oral administration, and BUD, 6 β OHBUD, 16 α -hydroxy-prednisolone (16 α OHPRED) and 6 α -hydroxy-budesonide were characterised by comparison with reference materials (7). Moreover, twelve BUD metabolites were identified and their tentative structures were proposed based on mass spectrometric data (7). Second, the urinary excretion profiles of BUD metabolites were evaluated after oral and INH administrations to identify the best criterion to discriminate between authorized and forbidden administrations of BUD (8). In that study, it was demonstrated that using a reporting level of 30 ng/mL of 16 α OHPRED, the main metabolite of BUD, false adverse analytical findings (AAFs) are detected after allowed INH administration. 6 β OHBUD was found to be the best discriminatory marker (8), and was implemented in

WADA regulations (6). The adoption of the new marker drastically reduced the number of AAFs reported by WADA accredited laboratories (9, 10).

In our previous work (8), the clinical studies performed consisted of oral and standard INH doses, and only male subjects were recruited for both treatments. Since INT and high-dose INH treatments of BUD are also widely prescribed (1), it is of utmost interest to study the urinary excretion profiles of BUD metabolites after these administration routes. Moreover, it is important to study the metabolism of BUD in female subjects in order to evaluate potential gender differences.

The aim of the present work was to provide more data about the metabolism of BUD to improve the detection of its systemic use in sports. For that purpose, the urinary excretion profile of BUD and the most important metabolites, 6 β OHBUD and 16 α OHPRD (Figure 1S, supplementary information), were studied after INT, high-dose INH and oral administrations to healthy male and female volunteers.

2 Experimental

2.1 Clinical studies

Two clinical studies that involved different administrations of BUD to healthy Caucasian subjects were performed (Table 1). (protocol codes IMIMFTCL/DACORSIN/2 and IMIMFTCL/DACORSIN/3). Ethical approval of the clinical studies was granted by the local Research Ethical Committee (*Comitè Ètic d'Investigació Clínica CEIC-Parc de Salut Mar*, Barcelona, Spain) and the Spanish Medicines Agency (EudraCT protocol numbers 2014-002862-54 and 2017-004839-37, respectively).

A total of sixteen healthy volunteers, twelve females and four males, were recruited (Table 2). All participants gave their written informed consent prior inclusion and underwent a general physical examination, routine laboratory test, urinalysis, and a 12-lead electrocardiogram with results within normal values. All volunteers declared not to consume drugs that could alter GCs metabolism such as steroids, prohormones, contraceptives or diuretics.

The first clinical study was a cross-over clinical trial where four males and four females received INT and high-dose INH treatments (Table 1). Wash-out periods of 10 days were carried out between treatments. First, volunteers underwent an INT therapy that involved the administration of a daily dose of 256 µg (two sprays of 64 µg in each nostril) (Rhinocort®, AstraZeneca, Spain) for three consecutive days. The daily dose was administered as a single dose on day 1 and as half dose every 12 h on days 2 and 3. Second, subjects received an INH treatment that consisted of a daily dose of 800 µg (Pulmicort Turbuhaler®, AstraZeneca, Spain) for three consecutive days. Finally, male volunteers underwent an INH treatment with a daily dose of 1600 µg (Pulmicort Turbuhaler®, AstraZeneca, Spain). In both INH therapies, doses were administered as

half dose every 12 h for three consecutive days. Urine collection periods were the same for all three treatments: pre-administration and after the first administration from 0-6 h, 6-12 h and 12-24 h on days 1, 2 and 3, and from 0-12 h and 12-24 h on day 4.

The second clinical study consisted of a single-arm trial, where a single oral dose of 3 mg (sustained release capsules, Entocord; AstraZeneca, London, United Kingdom) was administered to eight female volunteers (Table 1). The oral treatment administered was the same as the one used in our previous study with male volunteers (8). Urine samples were collected before administration and after administration from 0-4 h, 4-8 h, 8-12 h and 12-24 h on day 1, from 0-12h and 12-24 h on day 2, and from 0-24 h on days 3 and 4.

Urine samples were collected in sterile containers. Preliminary measurements, including specific gravity (SG) and pH, were performed. SG was determined using an Atago UG- α Refractometer (Atago, Tokyo, Japan), and pH was measured by a Crison GLP 21 pH meter (Crison, Alella, Spain). Urine samples were stored at -20°C until further analysis.

2.2 Chemical and Reagents

Budesonide (BUD) and ammonium formate (analytical grade) were obtained from Sigma (St Louis, MO, USA). 16 α -hydroxy-prednisolone (16 α OH-PRED), 6 β -hydroxy-budesonide (6 β OH-BUD), budesonide-22,23,23,24,24,25,25,25-d₈ (BUD-d₈), 6 β -hydroxy-budesonide-22,23,23,24,24,25,25,25-d₈ (6 β OH-BUD-d₈), and 16 α -hydroxy-prednisolone-11,12,12,21,21-d₅ (16 α OH-PRED-d₅) were purchased from Toronto Research Chemicals (Toronto, Canada). The β -glucuronidase preparation (type *Escherichia coli* K12) was purchased from Roche Diagnostics, GmbH (Manheim, Germany). Analytical grade disodium hydrogen phosphate, sodium hydrogen

phosphate, potassium carbonate, ethyl acetate, acetonitrile and methanol (liquid chromatography gradient grade), and formic acid (liquid chromatography-mass spectrometry grade) were obtained from Merck (Darmstadt, Germany). Milli-Q water was obtained using a Milli-Q purification system (Millipore Ibérica, Barcelona, Spain).

2.3 Sample Treatment

An extraction protocol previously described was used (8). Internal standards (ISTDs) (20 ng of BUD-d8, 40 ng of 16 α OHPRED-d5, and 40 ng of 6 β OHBUD-d8) were added to 2 mL of urine, followed by the addition of 500 μ L 1 M phosphate buffer, pH 7. Then, enzymatic hydrolysis was performed adding 30 μ L of β -glucuronidase enzyme from *Escherichia coli*, and the mixture was incubated for 1h in at 55°C in a water bath. The buffered solution was alkalized with 150 μ L of 25% potassium carbonate solution to pH 8-9, and the steroids were extracted with 6 mL of ethyl acetate. After centrifugation (5 minutes at 1400g), the organic layer was transferred into a new tube and evaporated to dryness under a nitrogen stream in a water bath at 40°C. The residue was reconstituted into 100 μ L of a mixture of water to acetonitrile (1:1, v/v).

2.4 LC-MS/MS analysis

Liquid chromatography-tandem mass spectrometry analyses were carried out using a triple quadrupole (Xevo TQ-S micro) mass spectrometer provided with an orthogonal Z-spray-electrospray interface (Waters Associates, Mildford, MA) coupled to an Acquity ultra-performance liquid chromatographic system (Waters Associates) for the chromatographic separation. Nitrogen and argon (both with purity $\geq 99.999\%$) were used as desolvation and collision gases, respectively. The desolvation and cone gas flows were set to approximately 1200L/h and 50 L/h, respectively. The nitrogen desolvation

temperature was set to 450 °C and the source temperature to 120 °C. A capillary voltage of 3.5 kV was used in positive ionization mode.

The liquid chromatographic separation was performed using an Acquity BEH C18 column (100 x 2.1 mm internal diameter, 1.7 µm particle size) (Waters Associates) using a column temperature of 45°C. Ammonium formate 1 mmol/L pH 3 (formic acid) (solvent A) and acetonitrile with formic acid (0.01%) (solvent B) were selected as mobile phase solvents. The percentage of organic solvent was linearly changed as follows: 0 min, 10% B; 0.5 min, 10% B; 7 min, 50% B; 8.7 min, 90% B; 8.8 min, 10% B; and 10 min, 10% B at a flow rate of 0.6 mL/min. The injection volume was 10 µL. Mass spectrometric analysis was performed in selected reaction monitoring mode by monitoring at least two transitions for each metabolite in the conditions described in Table 3.

2.5 Statistical Analysis

Statistical analysis was performed using computer software SPSS 12.0 (SPSS Inc., Chicago, IL, USA). Mann-Whitney U test was used to compare concentrations of BUD metabolites when two different INH doses were administered as well as when BUD was INT administered as a single dose or in two divided doses. The level of significance was established at $p < 0.05$.

In order to correct urinary concentrations by urine dilution, the concentrations were adjusted to a reference value of 1.020 following the Levine-Fahy equation (11, 12):

$$C_{SG} \left(\frac{ng}{mL} \right) = C \left(\frac{ng}{mL} \right) \times \left(\frac{1.020 - 1}{SG_{sample} - 1} \right)$$

Using the formula, SG-correction will lead to an increase of concentrations when samples present SG values lower than 1.020.

3 Results

6 β OHBUD, BUD, and 16 α OHPRED were measured in all samples collected after INT, INH and oral administrations. The sample preparation procedure applied allows for the detection of the sum of free and glucuronoconjugated metabolites. 16 α OHPRED was the main metabolite after all administrations studied, as reported previously (8).

3.1 Intranasal and inhaled administrations

Concentrations of 6 β OHBUD, BUD, and 16 α OHPRED obtained after INT and high-dose INH administrations are presented in Figure 1 and are also described in Table 1S of supplementary information. For INT and INH treatments, metabolites were detected on days 1 to 3, when subjects were under treatment, and were almost undetectable on day 4, when there was no administration. After INT treatment, 6 β OHBUD and BUD concentrations were very low (0.0-7.1 and 0.0-8.1 ng/mL, respectively). Concentrations obtained after INT administration did not show statistical differences when BUD was administered as a single daily dose or in two divided doses. After both high-dose INH treatments, concentrations of 6 β OHBUD, BUD, and 16 α OHPRED were of the same order. The majority of the samples collected after INH treatments presented 6 β OHBUD and BUD concentrations lower than 10 ng/mL (Figure 1), however some samples gave elevated concentrations (ranges 0.0-35.4 and 0.0-48.3 ng/mL for 6 β OHBUD and BUD, respectively) (Figure 1 and Table 1S). For 16 α OHPRED, high concentrations were obtained after both INT and INH treatments (2.6-66.4 and 3.4-426.5 ng/mL, respectively).

After both INT and INH treatments, higher concentrations were found in males than in females, especially for 6 β OHBUD and BUD (Figure 1 and Table 1S).

In order to assess the variability due to urine dilution, urinary concentrations were SG-corrected (Figure 2S, supplementary information). Post-administration urines presented SG values in the range of 1.003-1.030 and 1.004-1.023, for males and females, respectively. While SG-corrected concentrations of males were of the same order than concentrations obtained without correction, SG-normalization led to a much higher concentrations for females. Therefore, the gender differences previously observed were reduced when concentrations were normalized by SG.

The impact of SG-correction on the reduction of gender differences was more noticeable in the INH than INT samples, probably because 6 β OHBUD and BUD were not detected in some female samples collected after INT administration (LOQ 0.5 and 0.03 ng/mL for 6 β OHBUD and BUD, respectively (8)). Therefore, the effect of SG-adjustment could not be evaluated on those INT samples.

The urinary excretion profiles of 6 β OHBUD, BUD, and 16 α OHPRED after INT and high-dose INH administrations are presented in Figure 2. The profiles of the excretion rates obtained after INT and INH administration routes were very similar, however excretion rates were higher after INH administration. On days 1 to 3, excretion rates showed a consequent increase after the administration of each dose, and a rapid recovery in the post-administration hours. After the last dose on day 3, excretion rates linearly decreased, showing a complete elimination on day 4. Due to a high interindividual variability, clear gender differences were not observed in the excretion rates of BUD metabolites (Figure 2).

3.2. Oral administration

Concentrations of 6 β OHBUD, BUD, and 16 α OHPRED obtained after oral administration to females are presented in Figure 3 and are also described in Table 1S of

supplementary information. For comparison purposes, data from our previous study (8), where the same oral dose was administrated to males, are included.

Elevated concentrations were found only on day 1 (2.8-80.6, 1.5-36.1 and 10.4-532.2 ng/mL for 6 β OHBUD, BUD and 16 α OHPRED, respectively). In this time period, concentrations were higher in males than in females (Figure 3 and Table 1S).

Concentrations were corrected by SG in order to evaluate the variability due to urine dilution. SG values obtained in samples collected during the first day after oral administration, when BUD metabolites were mainly detected, were in ranges of 1.002-1.032 and 1.004-1.025 for males and females, respectively. After SG-normalization, gender differences were reduced because female concentrations considerably increased and concentrations of males were of the same order as the non-corrected ones (Figure 3S, supplementary information).

The urinary excretion profiles of 6 β OHBUD, BUD, and 16 α OHPRED after oral administration are depicted in Figure 2. Excretion rates increased and reached the maximum values in samples collected 0-8 h after administration, and linearly decreased till being completely eliminated in the third day. After oral administration, excretion rates of BUD metabolites were similar for females and males (Figure 2). The percentages of the dose of the different metabolites detected during the first 72h obtained in females were similar to those obtained for males (8), representing $1.4\pm0.9\%$, $0.7\pm0.3\%$, and $11.4\pm2.1\%$ of the dose for 6 β OHBUD, BUD, and 16 α OHPRED, respectively.

3.3. Discrimination between allowed and forbidden administrations

The suitability of the current WADA criterion to detect BUD misuse was evaluated. For that purpose, the percentage of AAFs detected using the reporting level of 30 ng/mL for 6 β OHBUD were calculated after different BUD administrations (Table 4A). Data obtained in a previous study that involved standard-dose INH treatment (400 μ g/day/3days) and oral administration to male volunteers were also included in the evaluation. As can be seen in Table 4A, false AAFs are detected after high-dose INH administrations because two male samples presented 6 β OHBUD concentrations higher than 30 ng/mL: One sample out of the 36 samples (3%) collected after INH 800 μ g/day treatment and one sample out of the 36 samples (3%) collected after INH 1600 μ g/day treatment with 6 β OHBUD concentrations of 35.4 and 31.0 ng/mL, respectively. BUD concentrations higher than 30 ng/mL were also found in two samples collected after high-dose INH treatments (Table 4A). One sample had concentrations of both 6 β OHBUD and BUD higher than 30 ng/mL. Therefore, a reporting criterion of 30 ng/mL for either 6 β OHBUD or BUD would lead to false AAFs after high INH doses (Table 4A).

Reporting levels that ensured no false AAFs after INH were proposed for each marker (Table 4A). The proposed reporting levels were 40, 50 and 450 ng/mL for 6 β OHBUD, BUD, and 16 α OHPRD, respectively, and they were chosen taking into account the maximum concentrations obtained after allowed INT and INH administrations (35, 48 and 427 ng/mL, respectively). Results showed that the best criterion to detect BUD misuse is a reporting level of 40 ng/mL for 6 β OHBUD (Table 4A), with no false AAF after INT and INH treatments and the highest number of AAFs after oral administration. Due to the fact that concentrations obtained after oral administration were lower for

females than for males, the percentage of AAFs detected for females is lower (Table 4A).

In order to assess the possibility to establish a reporting level based on SG-normalized concentrations, the percentage of AAFs obtained using reporting levels based on SG-corrected concentrations were calculated (Table 4B). Since concentrations increased after SG-correction, the reporting levels to avoid false AAFs after INT and INH administrations had to be higher. The maximum SG-corrected concentrations obtained after INT and INH doses were 45, 44, and 426 ng/mL for 6 β OHBUD, BUD, and 16 α OHPRED, respectively. Therefore, the reporting levels proposed were 50, 50 and 450 ng/mL for 6 β OHBUD, BUD, and 16 α OHPRED, respectively. Thus, the current reporting criterion of 30 ng/mL for 6 β OHBUD produced a higher number of false AAF when SG-corrected concentrations are monitored (Table 4B). Five samples presented SG-corrected concentrations of 6 β OHBUD higher than 30 ng/mL after high-dose INH treatments (3 samples (8%) after INH 800 μ g/day treatment and 2 samples (6%) after INH 1600 μ g/day treatment). Results obtained showed that the detection of AAFs after oral ingestion is very similar between males and females (Table 4B).

Ratios between metabolites were also studied to look for differences between routes of administration, but they did not improve the detection of systemic BUD.

4. Discussion

In this work, the urinary excretion profiles of 6β OHBUD, BUD and 16α OHPRD following INT, high-dose INH and oral administrations are described. In all treatments, recommended therapeutic dosages were used (1, 2). INT and INH doses administered were the maximum dosages recommended in therapeutics. In general, INT and INH treatments prescribed in therapeutics are longer than the ones performed in the present study (1). However, the administration of BUD by either INT or INH routes for three consecutive days was enough to evaluate a potential drug accumulation, and the impact on anti-doping controls. On the other hand, the oral treatment was a short and low-dose therapy (2), which allowed the evaluation of the minimum number of AAFs that could be detected following oral administration.

As expected, excretion rates obtained after oral administration were higher than after INT and INH treatments (Figure 2). Excretion rates obtained after INH administrations were greater than after INT treatment probably because INH doses were much higher. Since no accumulation was seen after multiple INT or INH administrations for three consecutive days, the results obtained in the present study could be extrapolated to longer INT and INH treatments.

Results obtained in the present work confirms that 6β OHBUD is the best marker to discriminate allowed from forbidden administrations of BUD (Table 4A). Interestingly, 6β OHBUD and BUD concentrations obtained after INT and INH administrations were very similar (Figure 2), but 6β OHBUD was excreted in higher amounts than BUD after oral administration (Figure 3). For this reason, 6β OHBUD is a better discriminatory marker than BUD. However, using a reporting level of 30 ng/mL for 6β OHBUD false AAFs may be reported after high-dose INH administrations. A reporting level of 40

ng/mL for 6 β OHBUD was found to be the best discriminatory criterion (Table 4A). Although increasing the reporting level would suppose a lower detection of AAFs after oral use, it is of paramount importance to avoid the possibility of reporting false AAFs after INT and INH treatments due to the wide use of BUD using these administration routes for therapeutic purposes. Moreover, BUD has reduced systemic effects (1), and therefore, it is not reasonable to expect that athletes use oral BUD for doping purposes. Nonetheless, it has to be taken into account that the oral therapy administered was a short and low-dose treatment, and therefore, the likelihood of considering a sample as AAF when it is collected during or after a high-dose treatment should be higher. On the other hand, it is re-confirmed that the use of a reporting level of 30 ng/mL for 16 α OH-PRED is not adequate, since a large number of AAFs were detected after allowed INT and INH administrations.

The possibility to establish a reporting level based on SG-corrected concentrations was evaluated, however the discrimination between allowed and forbidden administrations of BUD worsened. On the one hand, a higher number of false AAFs were detected after INH using the current reporting level of 30 ng/mL for 6 β OHBUD (Table 4B). On the other hand, a lower number of male samples was considered as AAF after oral administration in comparison to the results obtained using the proposed reporting level of 40 ng/mL for non-corrected concentrations of 6 β OHBUD (Table 4B). Therefore, the recommendation is to use a reporting level based on non-corrected concentrations, even though it may present difficulties to consider as AAF a diluted urine collected after oral administration.

To our knowledge, it is the first time that the urinary metabolism of BUD is studied in women, and it was found that concentrations of BUD metabolites were lower in females than in males. Interestingly, SG values of the post-administration urines were lower in females than in males and, therefore, the gender differences were reduced after correcting concentrations by SG. A study involving a larger number of male and females subjects should be conducted in order to thoroughly study the effects of BUD administration on urine dilution, and to assess if these potential effects are gender-dependent.

In conclusion, the assessment of the BUD metabolism after high-dose INH treatment demonstrates that false AAFs may be reported using the current WADA criterion. For this reason, the recommendation is to increase the reporting level to 40 ng/mL for 6 β OHBUD to detect BUD misuse in order to reduce the risk of reporting false AAFs.

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Conflict of interest

The authors wish to disclose that there were no conflicts of interest associated with professional relationships, and that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Tables

Table 1. Clinical studies performed (see text for explanation).

study	type of study	n	sex	route	daily dose	urine collection ^a
1	cross-over	4/4	M/F	INT	256 µg x 3 days	24-hour total urine up to day 4
		4/4	M/F	INH	800 µg x 3 days	24-hour total urine up to day 4
		4	M	INH	1600 µg x 3 days	24-hour total urine up to day 4
2	single-arm	8	F	oral	3 mg	24-hour total urine up to day 4

a) Collection periods after administration are detailed in the text

Table 2. Subjects' characteristics.

study	sex	n	age (years)	height (cm)	weight (kg)	BMI (kg/m ²)
1	F	4	33±7	170±2	62±5	22±2
	M	4	22±2	181±4	76±10	23±2
2	F	8	34±10	164±5	61±13	23±5

Values are mean ± SD.

Table 3. Analysis conditions of 6βOHBUD, BUD and 16αOHPRED by LC-MS/MS.

Retention time (RT), monoisotopic mass (MM), precursor and product ions (m/z), and collision energy (CE). The first product ion indicated for each metabolite was used for quantification purposes. Cone voltage was set to 20 V for all transitions.

Metabolite	RT (min)	MM (Da)	Precursor ion	Product ion(s)	CE (eV)
16αOHPRED	3.30	376	377	226	26
				147	30
				159	28
6βOHBUD	4.65	446	447	339	14
				121	35
				171	35
BUD	6.35	430	431	147	24
				173	26
				323	12
16αOHPRED-d5	3.20	381	382	229	26
6βOHBUD-d8	4.55	454	455	339	14
BUD-d8	6.25	438	439	173	20

Table 4. Percentage of AAFs detected after intranasal treatment (256 µg/day/3days, INT), inhalation (400 µg/day/3days, INH 400; 800 µg/day/3days, INH 800; 1600 µg/day/3days, INH 1600) and oral administration (single dose of 3 mg). Subjects' sex (males, M; females, F) and time (hours, h; days, d) are indicated. In A, reporting levels are established based on non-corrected concentrations. In B, reporting levels are established based on concentrations normalized by SG.

Results of INH 400 and oral administration in males were obtained in a previous study (8).

AAFs (%) detected after administration of BUD by different routes											
Compound	Reporting level (ng/mL)	INT (1-3d)		INH 400 (1-3d)	INH 800 (1-3d)		INH 1600 (1-3d)	oral (0-8h)		oral (8-24h)	
		F	M		F	M		F	M ^a	F	M ^a
A	6βOHBUD	30	0	0	0	3	3	44	92	25	50
		40	0	0	0	0	0	38	69	19	44
	BUD	30	0	0	0	3	3	25	62	0	21
		50	0	0	0	0	0	6	31	0	6
	16αOHPRED	450	0	0	0	0	0	13	31	0	6
B	6βOHBUD	30	0	0	0	8	6	81	81	31	69
		50	0	0	0	0	0	44	44	19	31
	BUD	30	0	0	0	0	0	31	25	0	6
		50	0	0	0	0	0	56	31	0	6

a) Data from a previous study performed by our group (8)

Figure captions

Figure 1. Concentrations of 6 β OHBUD, BUD, and 16 α OHPRED after intranasal treatment (256 μ g/day/3days, INT) and inhalation (800 μ g/day/3days, INH 800; 1600 μ g/day/3days, INH 1600). Time (days, d) and subjects' sex (female, F; male, M) are noted. Dotted line corresponds to the reporting level of 30 ng/mL for GCs.

Figure 2. Urinary excretion profiles (ng/min, mean + SD) of 6 β OHBUD, BUD and 16 α OHPRED in samples collected after intranasal (INT), inhaled (INH) and oral administrations. Dotted line corresponds to females and full line corresponds to males. Arrows indicate administrations. Data included involve results from a previous study where BUD was orally administered to males (8).

Figure 3. Concentrations of 6 β OHBUD, BUD and 16 α OHPRED after oral administration. Time (hours, h) and subjects' sex (female, F; male, M) are noted. Dotted line corresponds to the reporting level of 30 ng/mL for GCs. Data included involve results from a previous study where BUD was orally administered to males (8).