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Metabolic profile of budesonide after different administration routes and doses

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Abstract

We have evaluated the excretion profile of budesonide and its main metabolites after oral and inhaled administration at different therapeutic doses and formulations, with the aim to select the best criteria to discriminate between the forbidden and the permitted use of budesonide. For this purpose, three caucasian healthy volunteers were treated with different pharmaceutical formulations and the urine was collected before and after a single administration at different time intervals until 48h. The estimation of the budesonide and its metabolic products was carried out by LC-MS/MS after a liquid/liquid extraction of the urine samples using the analytical procedure currently adopted by our laboratory to determine glucocorticosteroids in urine samples. Our data show that (1) the urinary level of 16α-hydroxy-prednisolone is higher than the reporting limit also after inhalation (2) the concentration of the parent compound and of the hydroxylated metabolites exceeds the reporting limit only after systemic administration (3) the ratio between the epimers of the parent compound might be used as additional information in the discrimination between systemic and permitted administration routes.

Introduction

Glucocorticoids are included since 2004 in the WADA List of Prohibited Substances and Methods due to their capacity to increase resistance during physical exercise, as well as to induce euphoric and ergogenic effects [1]. Their administration is forbidden “in competition” only in the case of administration by oral, intravenous, intramuscular or rectal routes. To discriminate between systemic and permitted administration, a reporting threshold has been set by the WADA at 30 ng/mL for the urinary concentrations of parent compounds or their metabolites [2]. Unfortunately, as already reported by different research groups, this strategy is not always valid [3]. An example is represented by budesonide, whose intake is currently revealed by the presence in urine of its main phase I metabolite, 16α-hydroxy-prednisolone.

Experimental

Budesonide, 16α-hydroxy-prednisolone, 6β-hydroxy-budesonide, their corresponding deuterated compounds, and 6α-hydroxy-budesonide were supplied by Toronto Research Chemicals (Canada). All chemicals were from Sigma-Aldrich Italia S.p.A. (Milano, Italy). Different preparations of oral and inhaled budesonide at different dosages were administrated to 3 caucasian healthy volunteers (volunteer 1: female 26 years; volunteer 2: female 30 years; voluneer 3: male 32 years), using common drug delivery devices for patients treatment (Figure 1a). Urine samples were collected before administration and after 2, 5, 8, 12, 20, 24, 28, 31, 34, 44, 48 hours. Written consents were obtained from patients allowing the use of urines for research purposes. To 3 mL of urine, 100 ng/mL of 17α-methyltestosterone and 30 ng/mL of 6β-hydroxy-budesonide-d_8, budesonide-d_8 and 16α-hydroxy-prednisolone-d_5 were added. Enzymatic hydrolysis was carried out at pH 7.4 for 1 hour at 55°C using β-glucuronidase from *E. coli*. Then, the analytes were extracted with 7 mL of methyl *tert*-butyl ether and the organic layer was transferred and evaporated to dryness and reconstituted in 50 µL of mobile phase. 20 µL were then injected in the LC-ESI-MS/MS.

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For LC analyses an Agilent 1200 Rapid Resolution Series HPLC pump with binary gradient system (Agilent Technologies S.p.A, Cernusco sul Naviglio, Italy) was used. The chromatographic separation was performed using a Supelco Discovery C18 column (2.1x150 mm, 5 µm). The mobile phases used were: water (eluent A) and acetonitrile (eluent B), both containing 0.1% (v/v) formic acid. The flow rate was set at 250 µL/min and the gradient program used is reported in Figure 1b.
Mass spectrometry experiments were carried out using an Applied Biosystems (Applera Italia, Monza, Italy) API4000 instrument with electrospray ion source in positive mode at 500°C, applied capillary voltage set at 5000V. Tandem mass spectrometry in selected reaction monitoring acquisition mode (SRM) was employed for analysis. Parameters for budesonide and its metabolites are reported in Figure 1c. For the identification and quantification of the analytes it was used the analytical method validated and currently adopted by the anti-doping laboratory of Rome to confirm the presence of budesonide and related metabolites in human urine.

Study	1	2	3	4
Drug delivery device	Capsules	Turbohaler	Metered-Dose Inhaler (MDI)	Aerosol
Formulation	Capsules Intesticort®	Powder Pulmax®	Suspension Aircort®	Suspension Aircort®
Route of admin.	**Oral**	**Inhaled**	**Inhaled**	**Inhaled**
Dose	3 mg	200/300 µg	400 µg	1 mg
Administration	single	single	single	single
Volunteers	2 females 1 male	2 females 1 male	2 females 1 male	2 females
Urine collection	48 h	48h	48h	48h

1a.

Time (min)	% B
0.0	10
7.0	40
13.0	60
14.0	100

1b.

Compound	Rt (min)		Q1 (m/z)	Q3 (m/z)	CE (eV)
16α-OH-pred.lone	10.35		377.3	359.6, 341.5	20,20
6α-OH-bude(*22R/22S*)	11.46	11.66	447.0	339.0, 171.0	25,35
6β-OH-bude(*22R/22S*)	12.31	12.44	447.0	339.0, 171.0	25,35
Bude(*22R/22S*)	15.41	15.62	431.0	413.0, 323.0	25,25

1c.

Figure 1: a) *In vivo* studies protocols; b) gradient program for LC setting; c) LC-MS/MS parameters for budesonide and its metabolites.

Results and Discussion

Commercially available pharmaceutical formulations contain a C22 epimeric mixture of budesonide. After both systemic and inhaled administration, budesonide undergoes extensive phase I metabolism and the most characteristic metabolites are 16α-hydroxy-prednisolone, stereo-selectively formed only from 22R-budesonide, 22R-/22S-6β-hydroxy-budesonide and 22R-/22S-6α-hydroxy-budesonide (Figure 2, Table 3). Since the early elimination phase of the drug, 16α-hydroxy-prednisolone is the most excreted metabolite and this trend is confirmed till the end of the elimination.

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The urinary levels of the analytes after budesonide administration through different routes and at different dosages were as follows:

- after oral administration, 16α-hydroxy-prednisolone, budesonide, 6β-hydroxy-budesonide and 6α-hydroxy-budesonide concentrations significantly exceeds the WADA reporting threshold of 30 ng/mL for at least 12 hours respectively (Figure 2, Table 1). Within 20 hours after administration, high values of the epimeric ratio between 22S- and 22R-budesonide were determined (Figure 3);
- after inhaled administration, only the 16α-hydroxy-prednisolone exceeds the WADA reporting threshold. Instead, 6β-hydroxy-budesonide, 6α-hydroxy-budesonide and budesonide do not exceed this threshold. Within 20 hours after administration, low values of the epimeric ratio between 22S- and 22R-budesonide were measured. In the case of inhaled administration using the MDI device, the concentrations of budesonide and metaboites strongly depend on the percentage of the dose that is swallowed.

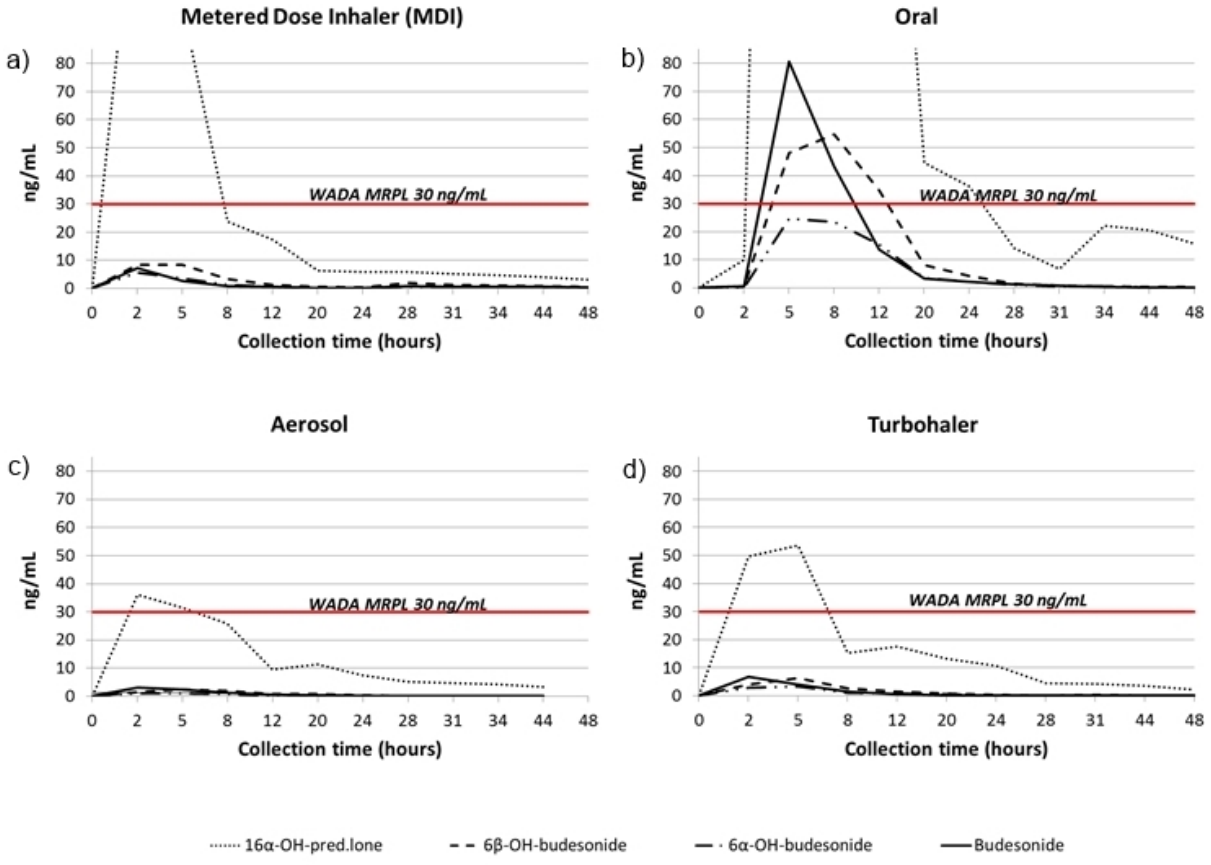

Figure 2: Excretion profile of budesonide, 16α-hydroxy-prednisolone, 6α/β-hydroxy-budesonide in urine collected from volunteer 1 after single administration of a) Aircort® MDI (400 µg); b) Intesticort® capsule (3mg); c) Aircort® aerosol (1mg); d) Pulmax® turbohaler (200/300 µg).

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Compound	Time (h)	Oral (ng/mL)			Metered Dose Inhaler (ng/mL)			Turbohaler (ng/mL)		
		Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 1	Volunteer 2	Volunteer 3
Budesonide	2	*	*	230	7.1	*	24	6.6	8.1	5.3
	5	81	77	370	*	5.1	21	*	*	5.3
	8	43	17	89	*	*	5.5	*	*	*
	12	14	8.1	47	*	*	*	*	*	*
	20	*	*	21	*	*	*	*	*	*
16α-hydroxy-prednisolone	2	9.9	21	720	**150**	**60**	**230**	**50**	**52**	29
	5	590	780	710	**110**	**140**	**180**	**54**	**80**	**110**
	8	340	390	140	24	23	**59**	15	**63**	13
	12	260	180	95	17	22	15	18	25	*
	20	45	59	17	6.3	15	**82**	13	11	*
6α-hydroxy-budesonide	2	*	*	79	5.6	*	10	*	*	*
	5	27	25	180	*	5.4	11	*	*	6.5
	8	23	19	53	*	*	5.6	*	*	*
	12	15	10	59	*	*	*	*	*	*
	20	*	*	14	*	*	5.9	*	*	*
6β-hydroxy-budesonide	2	*	*	110	8.2	*	16	*	*	*
	5	48	41	220	8.3	8.5	21	6.2	6.3	8.7
	8	54	39	120	*	*	14	*	*	*
	12	34	24	110	*	*	*	*	*	*
	20	8.2	12	41	*	*	14	*	*	*

True positive result False negative result False positive result

Table 1: Concentrations of budesonide, 16α-hydroxy-prednisolone and 6α/β-hydroxy-budesonide, up to 20 hours after administration, adjusted for specific gravity. Each value represents the mean of three independent determinations.
* Concentrations below the limit of quantitation (LOQ) of the analytical method (LOQ = 5 ng/mL for budesonide, 16α-hydroxy-prednisolone and 6α/β-hydroxy-budesonide).

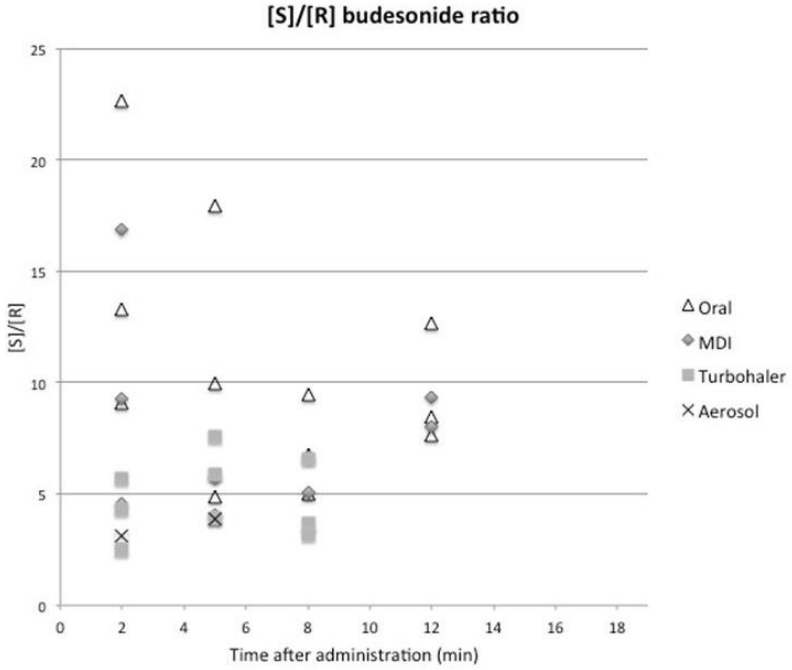

Figure 3: In vivo urinary epimeric ratios for budesonide up to 20 hours after oral and inhaled administration at different therapeutic doses and formulations, using different drug delivery devices.

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Conclusions

- The detection of 16α-hydroxy-prednisolone as marker of budesonide abuse can frequently lead to misinterpretation, with the risk of false positive results, in agreement with data reported by previous studies [3,4] (Figure 2, Table 1).
- On the other hand, 6β-hydroxy-budesonide and budesonide exceed the reporting limit only after systemic administration and could be selected as more appropriate diagnostic markers to better discriminate between the permitted and the forbidden intake (Figure 2, Table 1).
- High values of the ratio between the epimers of the parent compound are related to the systemic administration (Figure 3) and might be used as additional information, as already reported for salbutamol [5]. Further studies involving more volunteers and doses will be performed to assess the reliability of these results.

References

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