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Is budesonide administration by inhalation really permitted?

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Abstract

Budesonide is a glucocorticosteroid which possesses a high ratio of topical to systemic activity as a result of extensive first-pass metabolism to metabolites of minimal activity. According to WADA Prohibited List budesonide is prohibited only when administered by oral, intravenous, intramuscular or rectal route. Since administration of budesonide by inhalation or intranasal is not prohibited, it is rather not feasible to obtain TUE for its use by this routes. To differentiate whether glucocorticosteroids were administered by one of the prohibited routes or not, a specific reporting limit for urinary concentrations of parent compounds and their respective metabolites was established at 30 ng/mL. The results of five controlled administration studies performed in the Warsaw laboratory in the years 2011- 2012 show that administration of budesonide by inhalation at therapeutic doses may lead to appearance in urine of 16alpha-hydroxyprednisolone, the main budesonid metabolite, at concentrations greater than the reporting limit.

Introduction

Corticosteroids are drugs with anti-inflammatory properties used for treatment of variety of conditions, including allergic diseases, brain tumors, and many others. The possible desired effects for sportsmen abusing these substances are pain alleviation, euphoria, reduction of fatigue perception, all of which may enhance performance. In agreement with the WADA Prohibited List [1], all corticosteroids are prohibited when administered by oral, intravenous, intramuscular or rectal route. To differentiate whether corticosteroids were administered by one of these routes or not, a specific threshold for urinary concentrations of parent compounds and their metabolites was established at 30 ng/mL. Budesonide is a substance belonging to the corticosteroid class which possesses a high topical activity with reduced systemic side effects as a result of the extensive first pass hepatic clearance and therefore, low oral bioavailability [2]. Budesonide is mainly used by inhalation in treatment of asthma and by nasal administration in allergic rhinitis [3]. It is also used by oral route for treatment of the inflammatory bowel disease [4]. The aim of this study was to investigate whether administration of budesonide by non-prohibited routes (intranasal administration or inhalation) can lead to a concentration in urine of its main metabolite, 16alpha-hydroxyprednisolone, greater than 30 ng/mL.

Experimental

Urine samples were collected from five athletes during controlled excretion studies. The athletes took the drug in accordance with the treatment course (dose, frequency, route of administration) declared in the doping control forms. Four athletes administrated Symbicort Turbuhaler by inhalation. The first took a single dose of 320 µg of budesonide, the second 320 µg of budesonide twice (the time difference between applications - 22h), the third a double dose of total 640 µg at once (Figure 1A). The fourth administrated 640 µg of budesonide three times (the time difference between applications 10 h and 9h, respectively) (Figure 1B). The fifth athlete administrated Buderhin by intranasal route (100 µg of budesonide). Moreover, athletes exercised to reproduce conditions of post-competition sample collection. Urines were collected at different time-points up to 9 hours post-administration.
Sample preparation was as follows: 3 mL of urine was spiked with mefruside (IS, final conc. of 30 ng/mL), and the pH was adjusted to 7 with a phosphate buffer (0.8M) followed by addition of 50 µL of β-glucuronidase. The hydrolysis was carried out for 1h at 50°C, and then 1 mL of 20% K_2CO_3/$KHCO_3$ buffer and 6 mL of MTBE were added. Next, the organic phase was

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evaporated and the dry residue was reconstituted in 80 µL of mobile phase. Calibrators containing 16alpha-hydroxyprednisolone at concentrations of 10, 30, 60, 120, 240, 360 ng/mL with mefruside as internal standard were prepared using blank urine (pooled). Chromatographic separation was carried out on a Waters Alliance 2695 system equipped with a Thermo Hypercarb column (100 mm × 2.1 mm, 5 µm) and a Hypercarb precolumn. The mobile phase consisted of 0.5% acetic acid in water (A), 0.5% acetic acid in acetonitrile (B), and 0.5% acetic acid in isopropanol (C). A step-wise LC gradient was employed (Table 1) at a constant flow rate of 400 µL/min at 58°C. Samples were stored at 10°C in the autosampler prior to analysis and the injection volume was fixed at 10 µL. MRMs of the studied substances were analyzed with a Micromass Quattro Micro API mass spectrometer (Waters) equipped in an ESI source. The desolvation gas flow was set at 600 L/h at a temperature of 350°C and the source temperature was 120°C. The cone flow was set at 40 L/h. Capilarry was set to 3.20 kV and all the following MRMs were traced in a positive mode:
16alpha-hydroxyprednisolone: cone 26V; MRMs: 377.20>146.90 (quantitation MRM; CE 20eV), 377.20>225.07 (CE 15eV), 377.20>323.27 (10eV), 377.20>359.13 (10eV); Mefruside: cone 25V; MRM: 382.97>129.05 (CE 20eV).
The peak area ratios between the transmission of the analyte and the transmission of the internal standard were plotted against the concentration. Using a least square fit, good linearity ($r^2 \geq 0.99$) was observed in the range of 10 - 360 ng/mL. The precision and accuracy of the method were tested at three levels 10, 30, 120 ng/mL in five repetitions. Three independent runs were performed on three different days. Repeatability (within-run) and reproducibility (between-run), expressed as the coefficient of variation (CV), were below the acceptable threshold of 20% for LOQ and 15% for the other QC samples [5]. Within-run and between-run accuracies defined as deviation of the mean measured concentration from the theoretical concentration for all compounds were within 20% of the nominal values for the LOQ and 15% for the other QC samples [5]. Selectivity was very good because interference from other monitored similar compounds (corticosteroids) could not be found. Analysis of 10 different blank control urine samples did not result in the detection of interfering substances, proving the specificity of the method. The limit of quantification of the method was 10 ng/mL and was defined as the lowest concentration where acceptable reproducibility and accuracy could be guaranteed (LOQ). The limit of detection was 5 ng/mL (defined arbitrarily as 1/2 LOQ).

Time	A%	B%	C%	curve
0.00	40	60	0	initial
2.00	40	60	0	constant
11.50	5	95	0	linear
12.50	5	5	90	linear
16.75	5	5	90	constant
18.00	5	95	0	linear

Table 1: LC Gradient employed in the study

Results and Discussion

The 2011 laboratory statistics published on the WADA website indicates that budesonide is the most frequent glucocorticosteroid identified in samples collected in competition [6]. In the years of 2011-2012, there were 13 adverse analytical findings of budesonide in Poland reported and in five cases, athletes (via NADO) requested a controlled excretion study.
Three out of four studies showed that levels of 16alpha-hydroxyprednisolone were higher than the reporting limit up to 12 hours after budesonide inhalation (Figure 1A). This is in agreement with already published data [7,8]. In one case, the athlete declared use of a total daily dose exceeding the maximal dose recommended by the drug manufacturer. Moreover, unusually high concentrations of 16alpha-hydroxyprednisolone suggested a possible manipulation during the study (Figure 1B); concentrations measured were in a range of those published for samples collected after systemic use [8]. Although it is worth noting that in this case the laboratory was not provided with a sample collected before the administration. Nasal administration of budesonide also resulted in the appearance of its main metabolite in urine; however the concentration did not exceed the reporting limit (Figure 1C).

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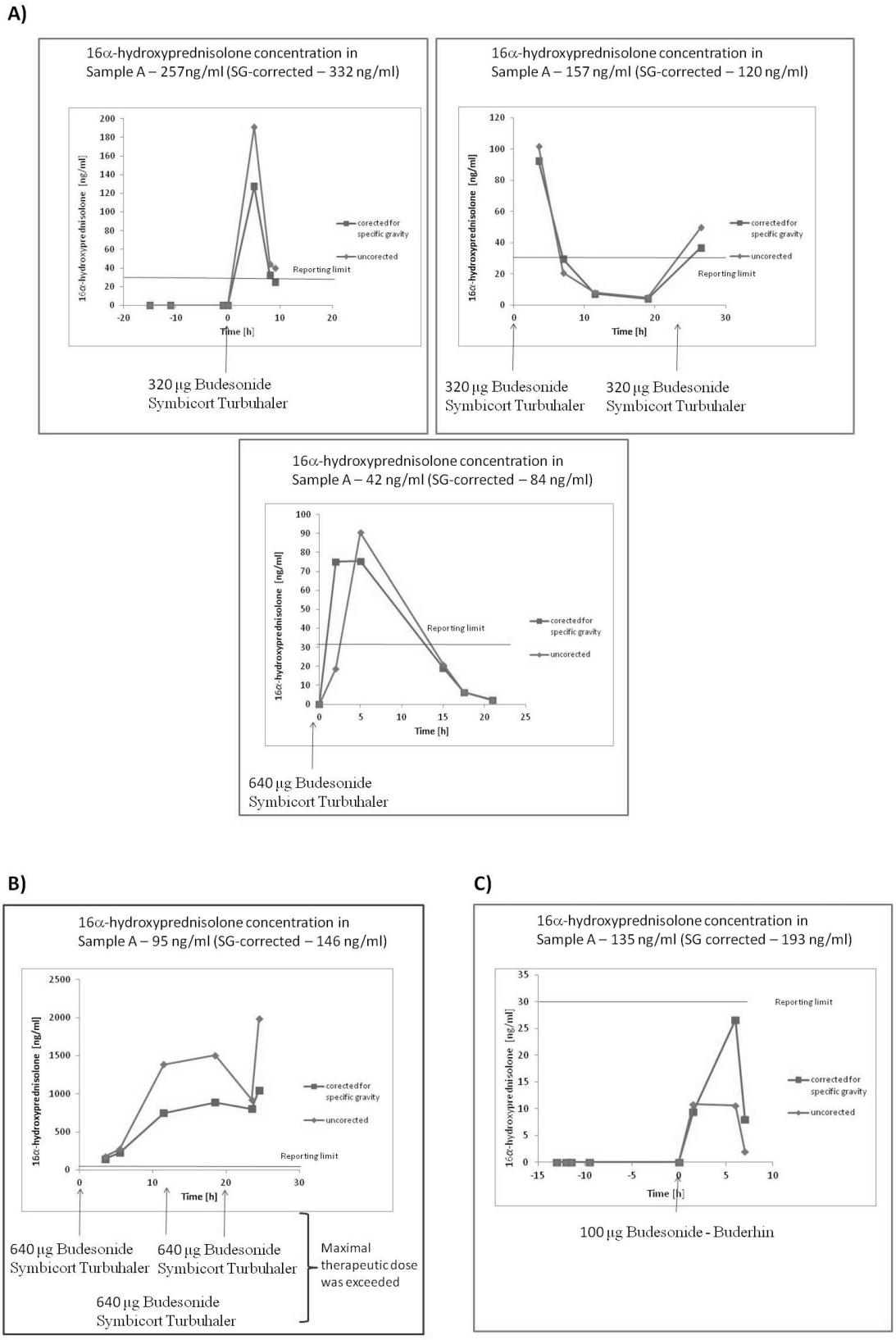

Figure1: Experimental data obtained for controlled studies. Urinary excretion profiles of 16 alpha-hydroxyprednisolone after administration of budesonide: by inhalation (A), inhalation and probably systemic (B), intranasal (C). Concentrations of 16 alpha-hydroxyprednisolone measured in the respective "A" samples collected in-competition are provided on the top of each graph.

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Nevertheless, the level of 16alpha-hydroxyprednisolone after 5 hours post-administration was very close to the reporting limit. Analysis of routine samples performed in Warsaw laboratory in the years 2011 and 2012 showed that about 35% of the samples containing 16alpha-hydroxyprednisolone exceeded the reporting limit. Moreover, none of the samples reported as AAF contained 16alpha-hydroxyprednisolone at concentrations clearly suggesting budesonide systemic use (Figure 2). To solve the problem, it is necessary to search for a specific marker of budesonide abuse by prohibited routes of administration. Indeed, it was postulated in a recent publication to measure concentration of 6beta-hydroxy-budesonide and use the reporting limit of 20 ng/mL to discriminate between forbidden and authorized administration [9].

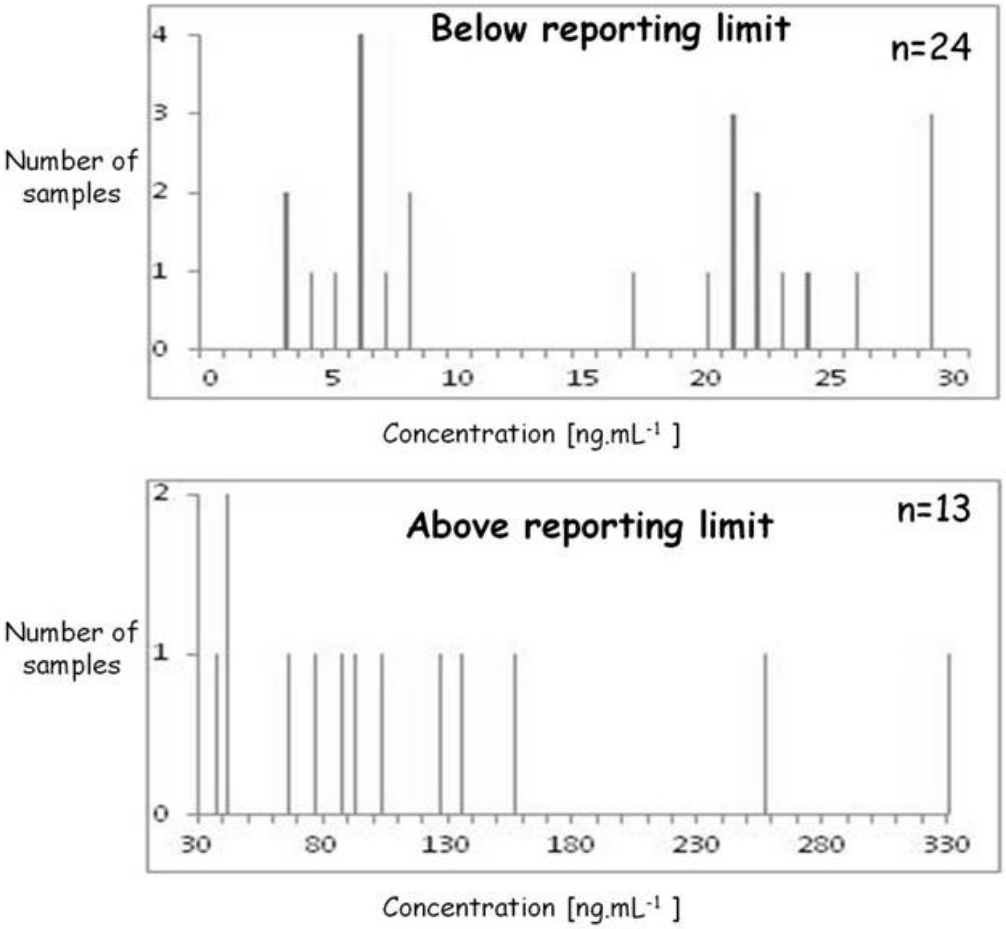

Figure 2: Distribution of 16alpha-hydroxyprednisolone concentrations in routine in-competition samples in the years 2011-2012

Conclusions

Altogether these results indicate that the use of budesonide by inhalation within 12 hours before and during competition may lead to a positive result of anti-doping testing. The only way to prove that budesonide had been taken by a non-prohibited route is a controlled excretion study. Only five out of thirteen athletes positively tested for budesonide decided to conduct such a study in Poland (years 2011/12). Possible reason for that may be a substantial cost of the controlled excretion study that, in Poland, is covered by the athlete.

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