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RECENT ADVANCES IN DOPING ANALYSIS (11)

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High amount of Salbutamol: A case report

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Introduction

Salbutamol, a β_2-agonist is forbidden in competition but it is authorized by inhalation with medical justification by the International Olympic Committee for the treatment of asthma and exercise-induced asthma in athletes [1]. Salbutamol may have stimulant effects (stimulation on the central nervous system) and inhalation of large doses of Salbutamol can have anabolic-like effect [2]. Salbutamol measured in urine is considered as stimulant when the concentration is higher than 100 ng/ml and it is considered as having anabolic properties when the concentration is higher than 1000 ng/ml (free plus glucuronide).

A few years ago some experiences made in our laboratory with healthy volunteers had shown that the concentrations of Salbutamol can be higher than 1000 ng/ml with therapeutic dose of Ventolin® nebulizer (data not published).

In a urine sample from a male (22 years old) in track-and field (400m), high concentration of Salbutamol (about 8000 ng/ml) was detected in summer 2002. This athlete took regularly Ventolin® and corticoids (Symbicort®) because he suffered from an allergic and effort asthma. The athlete claimed that he took Ventolin® only before training and competition especially in Spring and Summer as his symptoms were aggravated. On the day of the race, the athlete took corticoids in the morning and Ventolin® about one hour before the race (3 inhalations) then 30 minutes before the race (3 inhalations) and 3 inhalations at the arrival and before the doping control.

It was decided with the Medical Commission of Swiss Olympic to perform some investigations with this athlete in order to reproduce the excretion of high concentration of Salbutamol before considering the A-sample as a doping infraction with an anabolic agent.

Experimental

Protocol of the study

The protocol was performed during 4 days: the first day he was without medication and on days 2 and 3 the athlete took 3 times a day 3 inhalations of Ventolin® nebulizer. One inhalation of Ventolin® = 0.1 mg of Salbutamol.

Day	1	2	3	4
Treatment (Ventolin®)	-	3 inhalations	3 inhalations	-
Time	-	12h / 16h / 17h	12h / 16h / 17h	-
Symbicort® (corticoid)	-	-	+ (at 8h)	-
Urine collection	-	All urines	All urines	Morning urines

Samples preparation

The urines samples were prepared and analysed according to the anabolic agents procedures [3]. Free and glucuronide fractions were analysed

Derivatisation: The dry residues were derivatized with 50 µl of MSTFA/NH_4I/ethanethiol 1000:2:3 (v:w:v) for the glucuronide fraction and with 50 µl of MSTFA/TMSimidazole 1000:20 (v:v) for the free fraction.

Quantification

The concentration of Salbutamol was calculated using a single calibration point, 100 ng/ml for the free fraction and 1000 ng/ml for the glucuronide fraction.

GC-MSD analysis

The analyse were performed by Gas Chromatography coupled to Mass spectrometry (Agilent 5973N) in SIM mode for quantification.

Column: Zebron (5-ZB) 30m x 0.25 i.d., 0.25 µm film thickness.

Carrier gas: helium, head pressure: 15 psi.

Injection mode: splitless

Oven: 100°C for 1 min, then to 200°C at 16°C/min, then to 300°C (3min) at 3.8°C/min.

Results and Discussion

In this study, Salbutamol concentrations were examined after inhalation of a "normal" dosage. The aim was to reproduce what happened the day of the race and to see if it is possible to exceed the limit of 1000 ng/ml with inhalation.

In this protocol the dosage applied to the athlete wasn't so high as the day of the race (table 1): the highest concentration was reached 5-6 hours after the last inhalation of the first day and more rapidly the second day, after 3.5 hours of the last inhalation. On the competition day, there was about 2-3 hours between the race and the doping control. But the concentrations obtained were totally in accordance with the respective dosage.

Salbutamol is excreted in urine as a mixture of unchanged drug and its conjugated metabolites, mainly sulfate. In the lungs Salbutamol is not extensively metabolised and the proportion of metabolite after inhalation depends mainly on the percentage of the dose that is swallowed after impaction in the mouth and throat and absorbed from the gastrointestinal tract [4].

Table 1: Concentrations of free and glucuronide Salbutamol

Samples	Time in hours	T/E	Creatinine [mg/ml]	Conc Salbu free [ng/ml]	Conc Salbu (gluc.+ free) [ng/ml]	Salbu free/creat [ng/mg]	Salbu (gluc.+ free)/creat [ng/mg]
TG 1	0.00	0.9	0.53	0.00	0.00	0.00	0.00
TG 2	2.00	0.8	0.38	17.60	50.74	46.54	134.16
TG 3	3.50	0.9	0.44	63.85	287.16	143.81	646.84
TG 4	5.00	1.2	0.45	107.09	214.19	237.69	475.39
TG 5	5.75	1.3	0.33	99.45	345.98	299.40	1041.63
TG 6	6.25	1.4	0.15	41.10	111.83	271.67	739.13
TG 7	6.75	1.3	0.13	32.71	148.98	242.59	1104.78
TG 8	7.25	1.2	0.14	36.39	163.59	266.60	1198.46
TG 9	7.75	1.2	0.33	187.48	270.22	572.90	825.72
TG 10	8.75	1.2	0.64	202.71	**1206.04**	316.91	1885.47
TG 11	12.00	1.2	1.97	**661.61**	**3385.42**	335.86	1718.58
TG 12	12.75	1.2	0.89	221.80	710.52	247.97	794.32
TG 13	22.00	0.8	2.08	261.36	**1734.71**	125.45	832.65
TG 14	24.75	0.8	1.04	54.53	487.25	52.64	470.36
TG 15	25.50	0.7	0.32	37.81	140.37	116.72	433.31
TG 16	26.00	0.9	0.14	28.55	33.52	199.60	234.32
TG 17	27.00	1.0	0.27	42.94	73.81	158.28	272.06
TG 18	29.50	0.8	0.82	97.54	809.73	118.87	986.81
TG 19	33.00	0.8	1.83	**584.41**	**3181.80**	318.75	1735.42
TG 20	35.25	1.0	1.23	**526.10**	**1740.64**	428.87	1418.96
TG 21	36.75	1.0	0.73	163.76	821.71	223.79	1122.93
TG 22	43.00	0.8	1.07	152.29	748.38	142.05	698.05
Sample	***07.07.02***	***1.1***	***2.57***	***1318.98***	***8549.17***	***512.83***	***3324.00***

It was already showed that there are great differences in concentration between doses administrated orally or by inhalation: the concentrations of Salbutamol with oral intake are largely over 1000 ng/ml but that it is not reached with inhalation [5]. The concentration of free Salbutamol excreted in urine was also proposed to differentiate the two administration [6]: after oral administration free Salbutamol is greater than 500 ng/ml, whereas these concentrations are in general lower than those values with inhalation. But this marker is not sufficient as it was already observed. In our case free Salbutamol reached and overpassed 500 ng/ml [figure 1]. Reported to creatinine, the concentrations of free and glucuronide Salbutamol were affected and some concentrations were still high [figure 2].

Figure 1: Urinary Salbutamol (free and glucuronide ng/ml) as a function of time.

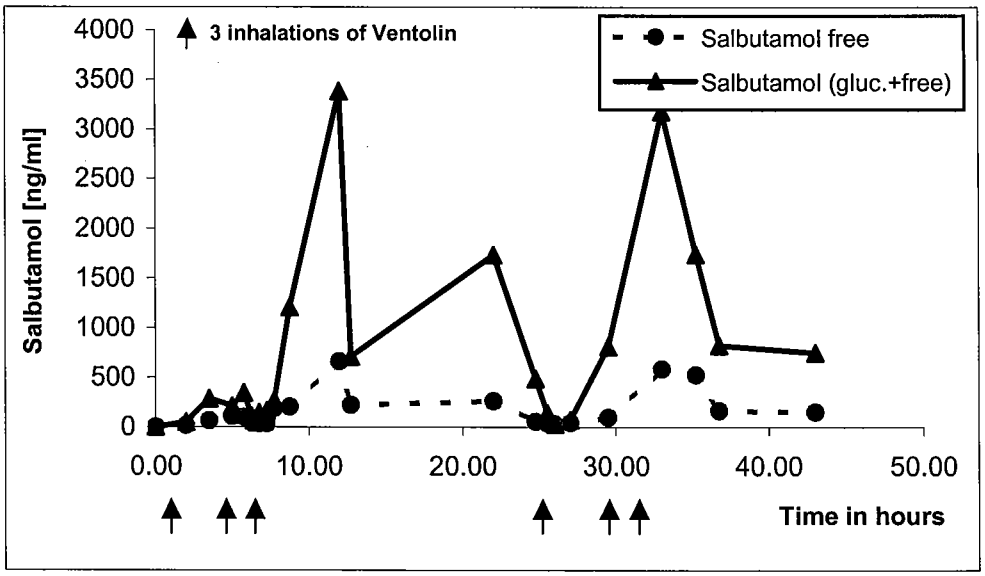
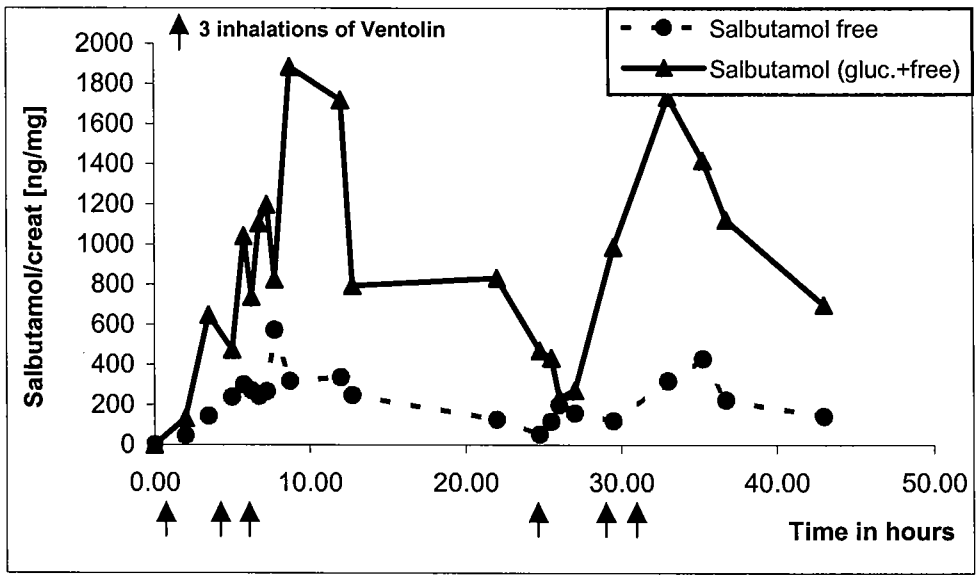


Figure 2: Urinary Salbutamol/Creatinine as a function of time.



Conclusion

This experience showed that Salbutamol aerosol application in a therapeutic dosage (or close to) can lead to a concentrations of non–sulphated Salbutamol in urine over 1000 ng/ml.
The concentration of non-sulphated Salbutamol was significantly higher to what was found during the protocol: this is certainly due to a more frequent intake of Ventolin® around the competition.
It should be determined if the frequent intake of Ventolin® is compatible with the status of top level athlete. A warning against Salbutamol "abuse" in case of asthma therapy can be made to the athletes and the doctors.
In this case the Swiss sanctioning body concluded that there was not a misuse of an anabolic agent but an overuse of a stimulant and sanctioned the athlete with a warning.

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