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Ciclesonide (Alvesco®) – a new glucocorticosteroid for inhalative treatment

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SUMMARY

Since January 2005 Ciclesonide [CIC], a new, nonhalogenated inhalative glucocorticoid to treat the inflammation associated with persistent asthma is marketed. The respective formulation Alvesco® is available for adults and should be applied once a day via inhaler. Ciclesonide is administered as inactive parent compound to the lung and is there converted to its active metabolite desisobutyryl-ciclesonide [des-CIC]. Des-CIC has a high affinity for the glucocorticoid receptor, comparable to budesonide and fluticasone propionate. It is rapidly converted by CYP3A4 into a series of polar metabolites in the liver. The pro-drug CIC as well as its active metabolite des-CIC have been implemented into the existing screening procedure for glucocorticosteroids in human doping analysis. Studies regarding specificity, limit of detection, recovery, and robustness have been performed for the detection of CIC and des-CIC in human urine and satisfactory results were obtained.

INTRODUCTION

In human doping analysis requirements for the detection of inhalative glucocorticosteroids are focused on the most commonly prescribed glucocorticosteroids for the treatment of asthma. These formulations are budesonide [BUD], beclomethasone-17,21-dipropionate [17,21-BDP] and fluticasone-propionate [FP]. Since January 2005 a new, nonhalogenated inhalative glucocorticoid CIC has been marketed by Altana Pharma (Konstanz, Germany). The respective formulation Alvesco® is available for adults at three different concentrations: one single dose of 40, 80 or 160 µg should be applied once a day via a hydrofluoroalkane metered-dose inhaler.

All these agents (BUD, 17,21-BDP, FP, CIC) are chemically and structurally similar but have different pharmacodynamic properties (Dyer *et al*, 2006). CIC and 17,21-BDP are administered to the lung as inactive parent compounds, termed pro-drugs, with little anti-inflammatory properties. The relative receptor affinity *e.g.* for 17,21-BDP is only 4%, for beclomethasone 6% and for 21-beclomethasone monopropionate with less than 1% compared to the active compound 17-beclomethasone monopropionate; relative receptor affinity = 100 (Daley-Yates *et al.* 2001). CIC requires cleavage by endogenous carboxyl esterases that create the active metabolite des-CIC (*Fig.* 1). It is concluded that the human metabolism of CIC is primarily catalysed by one or more esterases and by CYP3A4.

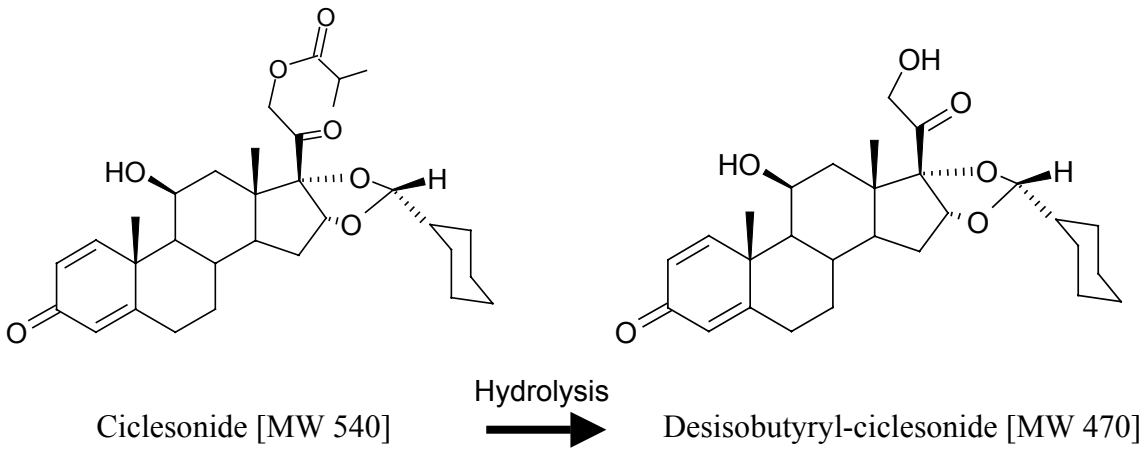

FIGURE 1: Conversion of CIC to des-CIC by hydrolysis in human lung tissue

Des-CIC has a high affinity for the glucocorticoid receptor, comparable to BUD and FP. It subsequently undergoes reversible esterification to fatty acids and forms various fatty acid conjugates at the carbon position 21 (Peet *et al.* 2005). Nave *et al.* (2006) confirmed that des-CIC oleate is the major fatty acid conjugate formed in human lung tissue. CIC and fatty acid conjugates of des-CIC are highly lipophilic. This may explain the high rate of absorption in lung tissue. Reversible fatty acid conjugation is acting as depot effect and supports the efficacy of one single dose daily.

Substances for inhalation like CIC, BUD FP and 17,21-BDP have been developed to minimize systemic adverse effects of glucocorticosteroids and to achieve low oral absorption and extensive first-pass metabolism by the liver. Small amounts of this four glucocorticosteroids, which may be swallowed while inhalation undergo an extensive first

pass metabolism in the liver, thereby reducing the amount of active drug that enters the systemic circulation and may cause serious side effects. Des-CIC is rapidly converted by cytochrome P450 3A4 into a series of polar metabolites, mainly by addition of two hydroxyl groups to des-CIC and some minor metabolites, *e.g.* 6-OH-des-CIC, 16α-hydroxyprednisolone and others as shown in *Fig.* 2 (Peet *et al.* 2005, Nave *et al.* 2006). 16α-Hydroxyprednisolone is formed at approximately one-tenth of the amount of M2 and M3

The benefits of the inhalation therapy of CIC may lead to abuse in human sports to enhance performance. It is therefore necessary to implement CIC and des-CIC and currently available metabolites into the existing and well established screening procedure for glucocorticosteroids in doping analysis.

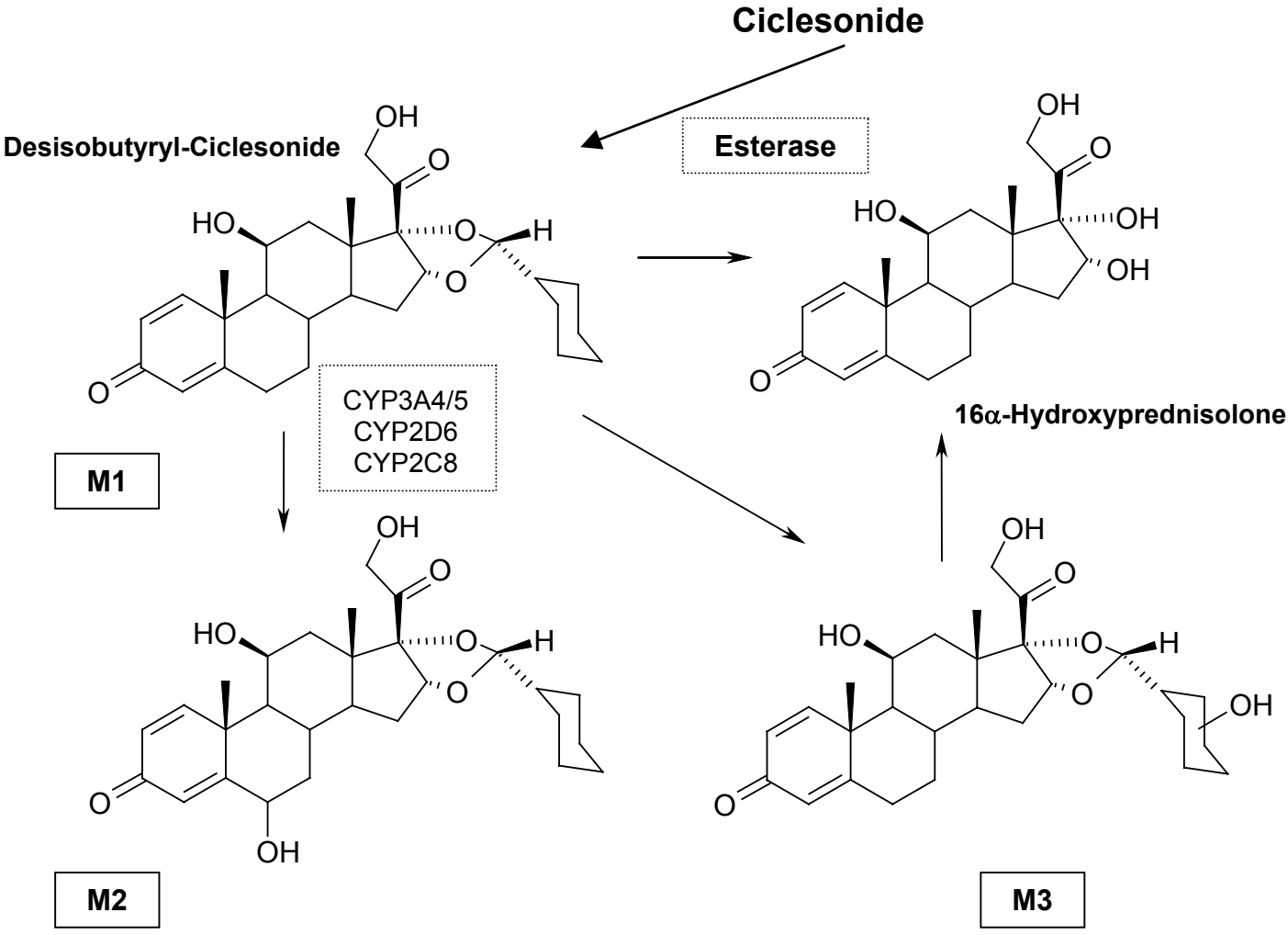

FIGURE 2: Proposed scheme for metabolism of Ciclesonide in man

MATERIALS AND METHODS

Chemicals

Ciclesonide, 16,17-[(Cyclohexylmethylene) bis (oxy)]-11-hydroxy-21-(2-methyl-1-oxopropoxy)pregna-1,4-diene-3,20-dione [11β, 16α (R)] and desisobutyryl-ciclosenide, 16,17-[(Cyclohexylmethylene) bis (oxy)]-11,21-dihydroxypregna-1,4-diene-3,20-dione [11β, 16α (R)] were kindly provided as reference material from Altana Pharma (Konstanz, Germany). Both substances are available for all WADA accredited laboratories upon request.

Sample preparation and analytical parameters

The sample preparation for glucocorticosteroid screening in human urine was performed as described in detail elsewhere (Mareck *et al.* 2004). Briefly, to 3 ml of urine the internal standard methyltestosterone is added and the pH-value of the urine is adjusted to 7 by adding 1 ml of 0.8 M phosphate buffer. After an enzymatic hydrolysis and liquid/liquid extraction with *tert*.-butyl methyl ether at pH 9.6, the organic layer is evaporated to dryness. The residue is resolved in methanol / ammonium acetate buffer (1:1) prior to LC/MS-MS analysis.

TABLE 1: Analytical parameters

Flow:	0.3 ml/min (splitless)
Solvents:	A: Ammonium acetate buffer (pH = 3.5, 5 mmol ammonium acetate, 1‰ glacial acetic acid) B: Acetonitrile
Gradient: Injection Volume:	10% Acetonitrile to 100% in 9 min 10 µl
Run Time / Post Time:	11 min / 3.5 min
Ion source: Interface Temperature: Ionisation Mode:	APCI 400°C Positive, multiple reaction monitoring of protonated molecular ions $(M+H)^+$: CIC: 541 (product ions: 523, 393, 323, 305) des-CIC: 471 (product ions: 453, 323, 341, 305)
Dwell Time:	40 msec

The analyses for glucocorticosteroids, screening and confirmation, are performed on a Hewlett Packard HP1100 liquid chromatograph coupled to a PE Sciex API 2000 triple quadrupole mass spectrometer. The column used is a Purospher Star RP-18e, 55 x 4 mm i.d.,

3 µm particle from Merck (Darmstadt, D). For screening purposes the LC and MS conditions are as listed in Table 1. The respective protonated molecules $(M+H)^+$, as well as the selected ions of CIC and des-CIC are chosen in accordance with their respective mass spectra (*Fig.* 3 and *Fig.* 4).

Method validation

Investigations regarding specificity, recovery, linearity, limit of detection and robustness of the applied method have been performed to detect CIC and des-CIC in human urine after enzymatic hydrolysis and liquid/liquid extraction with *tert*.-butyl methyl ether at pH 9.6.

RESULTS AND DISCUSSION

The pro-drug CIC and its active compound des-CIC have been successfully implemented into the existing screening procedure for glucocorticosteroids in human doping analysis. Satisfactory results for the investigations of specificity, limit of detection, recovery, linearity and robustness were obtained. Specificity of the method for the detection of CIC and des-CIC was accepted when no interfering substances at specific retention time were detected in 10 blank urine samples. The limit of detection, 1 ng/ml for CIC and des-CIC, was calculated by extrapolating a signal to noise ration of 3:1. For linearity spiking urine samples with CIC and des-CIC at 10, 20, 40, 60, 80 and 100 ng/ml respectively generated a six-point calibration curve. Linearity was found to be adequate for CIC and des-CIC (square fit, $r^2 \geq 0.999$), recovery was estimated with 73% for CIC and 80% for des-CIC.

According to the anti-doping regulations of the World Anti-Doping Agency [WADA], the administration of glucocorticosteroids via inhalation is permitted with a therapeutic use exemption (World Anti-Doping Agency, 2005 / 2006). WADA also set a reporting level at 30 ng/ml in urine for glucocorticosteroids. Due to the low therapeutically administered doses via inhalation most of the findings did not result in an adverse analytical finding. The sensitivity of the method was adequate to detect these substances in human doping control.

With the current applied LC-MS/MS method it is now possible to screen for CIC and des-CIC as well as for its minor metabolite 16α-hydroxyprednisolone. 16α-Hydroxyprednisolone is also main metabolite of budesonide and was therefore implemented into the glucocorticosteroid screening procedure longer time ago. For all substances the LODs fall far below the required performance limit of 30 ng/ml.

CONCLUSIONS

For human doping analysis, placebo controlled excretion studies with CIC should be performed to receive information about the urinary excreted metabolites and their respective amounts over time. All metabolites that are not commercially available (M2 and M3) have to be synthesized as reference material and implemented into the screening procedure for glucocorticosteroids.

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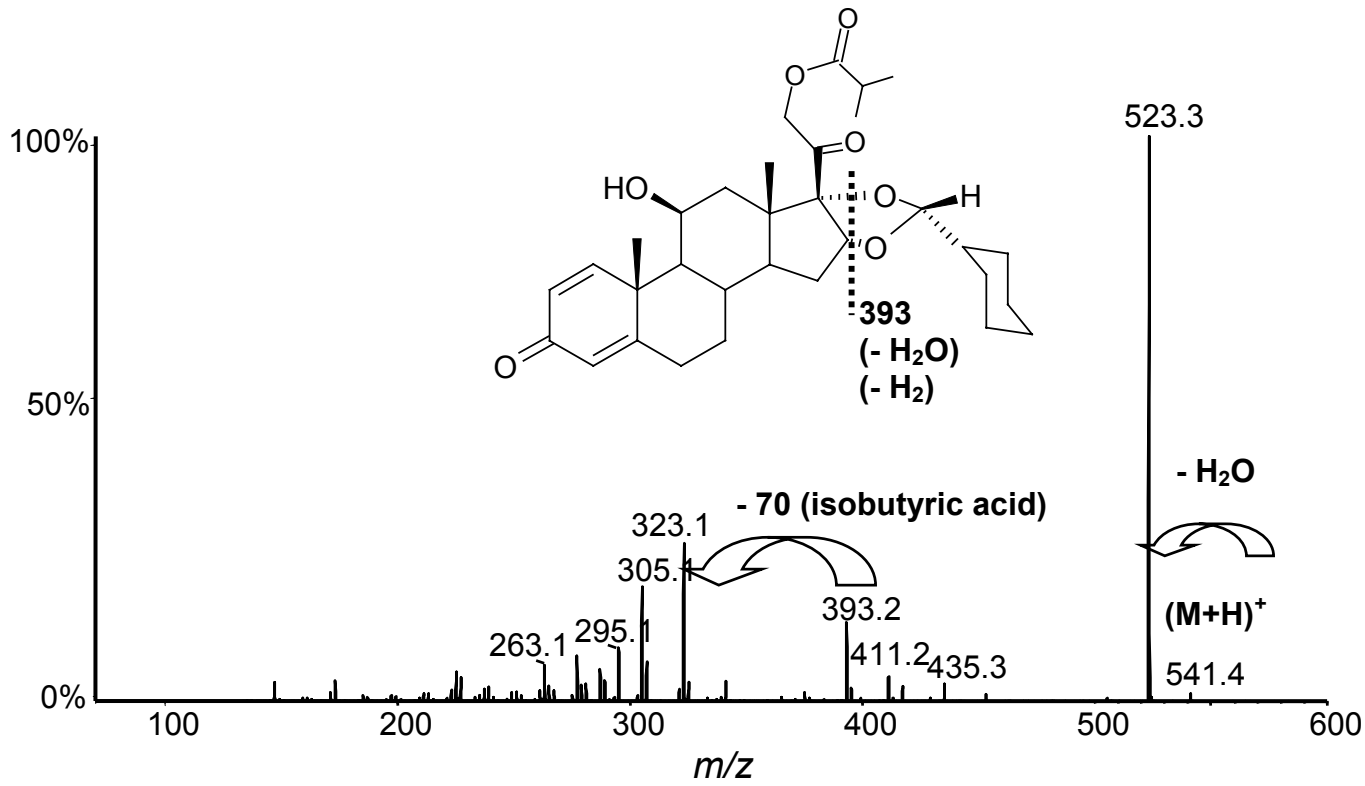

FIGURE 3: Product ion scan of the protonated molecule *m/z* 541 $(M+H)^+$ of ciclesonide

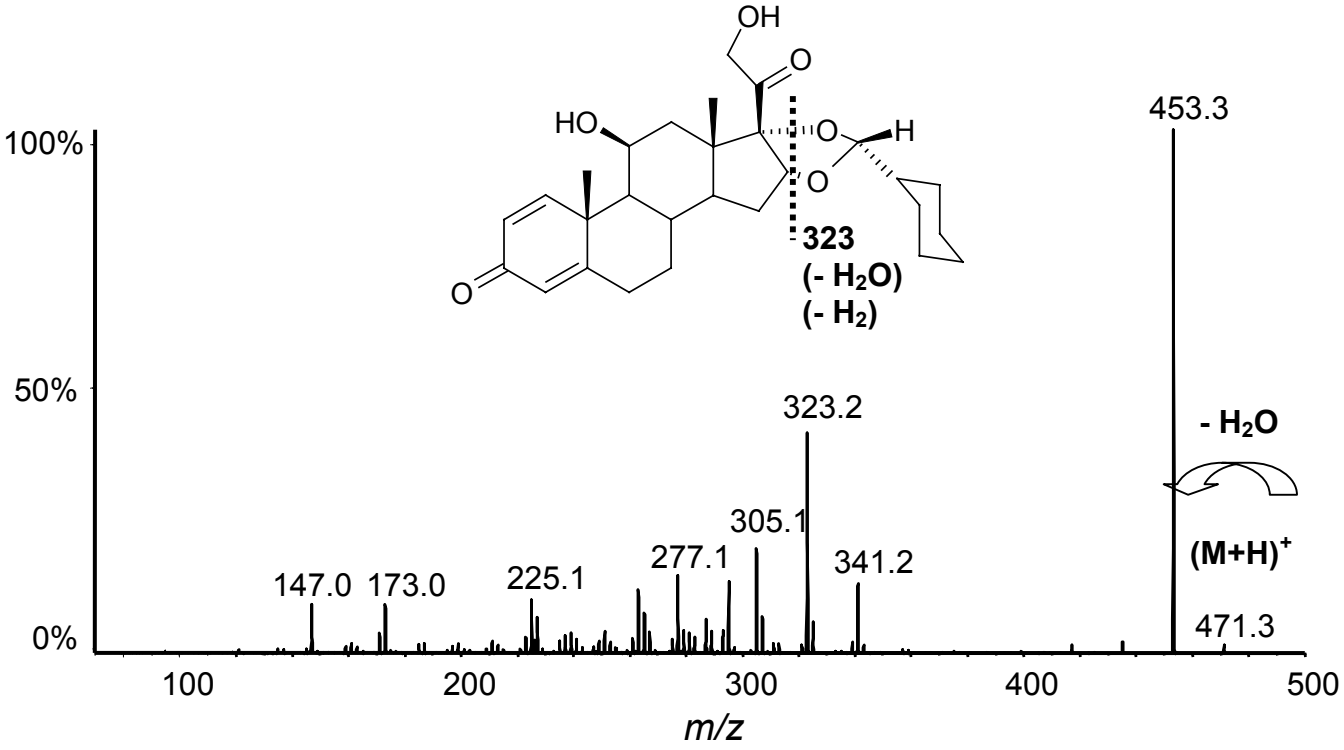

FIGURE 4: Product ion scan of the protonated molecule *m/z* 471 $(M+H)^+$ of desisobutyryl-ciclesonide