Journal of Steroid Biochemistry & Molecular Biology 138 (2013) 214–221

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Urinary profile of methylprednisolone and its metabolites after oral and topical administrations

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ARTICLE INFO

Article history:
Received 17 April 2013
Received in revised form 30 May 2013
Accepted 31 May 2013

Keywords:
Methylprednisolone
Doping
Routes of administration
Metabolites
LC–MS/MS

ABSTRACT

Methylprednisolone (MP) is prohibited in sports competitions when administered by systemic routes; however its use by topical administration is allowed. Therefore, analytical approaches to distinguish between these different administration pathways are required. A reporting level of 30 ng/mL was established for this purpose. However, the suitability of that reporting level for MP is not known. In the present work, excretion profiles of MP and different metabolites after oral and topical administrations have been compared. A method for the quantification of MP and the qualitative detection of fifteen previously reported metabolites has been validated. The method involved an enzymatic hydrolysis, liquid–liquid extraction and analysis by liquid chromatography coupled to tandem mass spectrometry. The method was found to be linear, selective, precise and accurate. The high sensitivity (limit of detection 0.1 ng/mL) and linear range (0.1–250 ng/mL) achieved allowed for the quantification of MP at both the low concentrations present after topical administration and the high concentrations detected after oral intake. The method was applied to samples collected after oral (4 or 40 mg) and topical administration (10 mg of MP aceponate/day for 5 consecutive days) to healthy volunteers. After oral administration, MP and all metabolites were detected in urines collected up to at least 36 h. Only MP and five metabolites were detected in samples obtained after topical treatment. As expected, concentrations of MP after topical administration were well below current reporting level (30 ng/mL), however 3 out of 4 samples in range 8–24 h after the low oral dose (4 mg) were also below that concentration. Taking into account metabolites detected after both administration routes, metabolites 16β,17α,21-trihydroxy-6α-methylpregna-1,4-diene-3,11,20-trione (M8) and 17α,20α,21-trihydroxy-6α-methylpregna-1,4-diene-3,11-dione (M11) are best markers to differentiate between topical and oral administrations. Their signals after topical administration were lower than those obtained in the first 48 h after all oral doses.

1. Introduction

Methylprednisolone (11β,17β,21-trihydroxy-6α-methyl-1,4-pregnadiene-3,20-dione, MP; Fig. 1) is a moderately potent glucocorticosteroid with anti-inflammatory and immunosuppressive effects [1]. It is used to treat a number of different conditions, such as inflammation (swelling), severe allergies, adrenal problems, arthritis, asthma, blood or bone marrow problems, eye or vision problems, lupus, skin conditions, kidney problems, ulcerative colitis, and flare-ups of multiple sclerosis. It works on the immune system to help relieve swelling, redness, itching, and allergic reactions. Topical MP demonstrates a low rate of percutaneous penetration [2,3] and an associated low incidence of local and systemic side-effects [3]. Applied once daily to affected skin, topical MP aceponate is rapidly effective and safe in the treatment of acute moderate to severe atopic dermatitis [4,5].

Performance enhancing effects of glucocorticosteroids in elite athletes are not clear [6,7], however some evidence of ergogenic effects and the health risks of using these compounds suggest to include them in the list of prohibited substances in sports of the World Anti-Doping Agency (WADA) [8]. The use of glucocorticosteroids in sports competitions is prohibited when administered by

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http://dx.doi.org/10.1016/j.jsbmb.2013.05.019

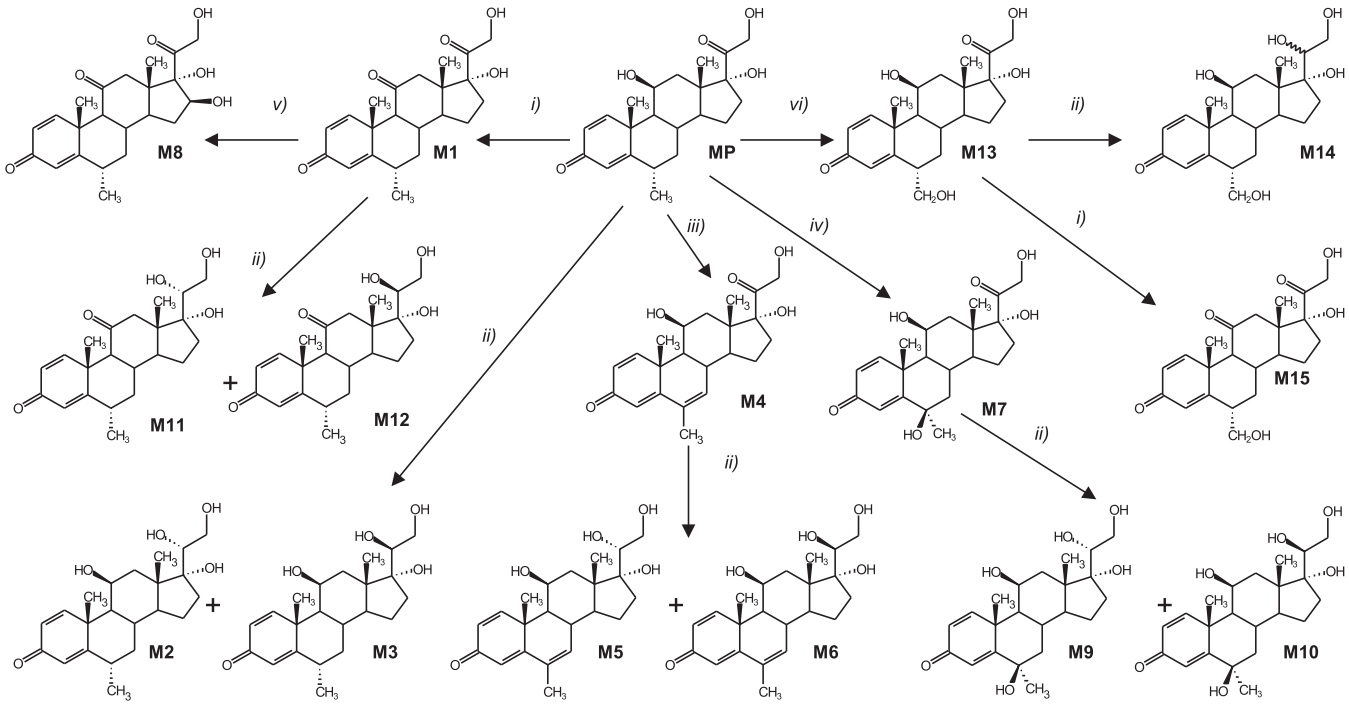

Fig. 1. MP and metabolites identified after oral administration in a previous study [22]. Metabolic pathways: (i) 11-oxydation, (ii) 20-reduction, (iii) 6,7-dehydrogenation, (iv) 6-hydroxylation, (v) 16-hydroxylation, and (vi) 22-hydroxylation.

oral, intravenous, intramuscular or rectal routes, but their use by other routes (e.g. topical) is allowed [9]. Therefore, strategies to discriminate between administration routes are needed to distinguish therapeutic treatments from forbidden use. In an attempt to make this distinction, WADA established an arbitrary reporting level of 30 ng/mL for glucocorticosteroids and their metabolites, as a general criterion [10]. Samples with lower concentrations should not be reported by the accredited laboratories because they are considered by WADA as a result of a therapeutic treatment.

However, it is known that this reporting level is not suitable for all corticosteroids for many reasons: wide range of therapeutic doses, different administration routes, different excretion rates in urine, ... For budesonide, the application of the reporting level to the main metabolite, 16α-hydroxy-prednisolone, results in a high percentage of false positive results after therapeutic inhaled administration, and the use of the unchanged drug, budesonide, as a marker results in a high percentage of false negative results after forbidden oral intake [11,12]. The analysis of several samples collected after oral and inhaled administrations led to the conclusion that the best compromise is the use of another metabolite, 6β-hydroxy-budesonide [12]. Up to our knowledge, the reporting level has never been evaluated for MP.

Differences in the excretion of metabolites in urine depending on the route of administration have been also encountered for other doping agents [13–15]. Therefore, the investigation of strategies to differentiate between administration routes requires the performance of metabolic studies to identify as many as metabolites as possible for each drug and the study of the excretion profiles of all the metabolites in urines collected after the administration of the drug using different routes. Several studies have been performed to identify MP metabolites in rats [16,17] and in humans [18–22].

In recent works, we comprehensively evaluated the metabolic profile of MP after oral administration using LC–MS/MS and GC–MS methods [20,22]. MP and fifteen metabolites were detected in urine (Fig. 1). The typical biotransformation pathways of glucocorticosteroids were seen: oxidation of the hydroxyl group in C11 position (M1), reduction of the C20 ketone group (M2 and M3) and combination of both (M11 and M12). Moreover, unsaturation (M4) or hydroxylation (M7) in C6 were also seen alone and in combination with C20-keto reduction (M5, M6 and M9, M10). Hydroxylation in C16 (M8) and C22 (M13) was also found, alone or in combination with other biotransformations (M14, M15). Due to the unavailability of reference standards, tentative structures of the metabolites were proposed based on mass spectrometric data. Metabolism after intra-articular and intramuscular administration was also described [21]. Eight phase I metabolites and six glucuronoconjugated metabolites were directly detected using LC–MS/MS techniques. Metabolites resulting from 11-oxidation, reduction of C20 and oxidation of the C20–C21 side chain were also detected and characterized using mass spectrometric data. However, excretion profiles of MP and metabolites after topical treatments have never been described and, in addition, few data on MP concentrations in urine after different treatments is available [23] to evaluate the reporting level defined by WADA.

The aim of the present work was to study the urinary profiles of MP and its metabolites after oral and multiple topical administrations in order to evaluate the discrimination threshold of 30 ng/mL defined by WADA regarding MP concentrations, and to detect other differences that could be used as effective criteria to discriminate between authorized and forbidden MP use. To fulfill this task a method for the quantitative analysis of MP and the qualitative analysis of fifteen metabolites in urine was developed and validated.

2. Material and methods

2.1. Chemical and reagents

Methylprednisolone (MP) was obtained from Sigma (St. Louis, MO, US). Methylprednisolone-9,11,12,12-d_4 (MP-d_4) was purchased from Toronto Research Chemicals (Toronto, Canada). The β-glucuronidase preparation (type *Escherichia coli* K12) was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Analytical grade di-sodium hydrogen phosphate, sodium hydrogen phosphate, potassium carbonate, ethyl acetate, acetonitrile