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Urinary excretion studies of meldonium after multidose parenteral application

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ABSTRACT

Meldonium is a drug exhibiting cardioprotective and anti-ischemic effects. Due to its potential performance-enhancing benefit in sports, meldonium was added to the World Anti-Doping Agency list of prohibited substances in 2016. Since then, a high number of adverse analytical findings reported on meldonium has questioned meldonium's detection time in urine. Hence, the objective of the current study was to characterize the pharmacokinetic urinary excretion pattern of meldonium when administered as multiple intravenous injections. Three injections of 250 mg meldonium were given over a time period of five days to six healthy volunteers and urine samples were collected for eight months after the last injection of the drug. For the quantification of meldonium in urine, a liquid chromatography-tandem mass spectrometry method was fully validated according to the World Anti-Doping Agency guidelines in terms of specificity, matrix interferences, intra- and inter-day precision, accuracy, carry-over, robustness, linearity, limit of detection, and limit of quantification. The assay was successfully applied to the pharmacokinetic study. A three-compartment model was found to best describe the pharmacokinetics of meldonium with average alpha, beta, and gamma half-lives of 1.4 h, 9.4 h, and 655 h, respectively. The detection time in urine varied between 94 and 162 days.

1. Introduction

Meldonium, also known as mildronate, is manufactured in Latvia and primary distributed in the Baltic countries and Russia. It is mainly used to treat cardiovascular disease including ischemia, as well as neurodegenerative disorders and bronchopulmonary diseases [1,2].

The mechanism of action involves a competitive inhibition of the enzyme λ-butyrobetaine hydroxylase, which converts λ-butyrobetaine in L-carnitine. Furthermore, the absorption and transport of L-carnitine is reduced by inhibition of the carnitine transporter carnitine/organic cation transporter type 2 (OCTN-2) [2,3]. The resulting decreased availability of carnitine in the cell leads to a shift in energy metabolism from fatty acid oxidation to increased glucose consumption, and as a consequence, ATP is generated more efficiently. This is beneficial in low oxygen conditions, like for instance in heart conditions where the cardiac muscle is deprived of oxygen [4]. For the same reason, meldonium might have performance enhancing effects in sports. Suggested benefits are amongst others a decrease in the production of lactic acid, the prevention of oxidative stress, enhanced endurance, and increased physical work capabilities [3]. Due to these effects, meldonium was added to the World Anti-Doping Agency's (WADA) prohibited list in 2016 and is classified as a metabolic modulator under section four [5].

In early 2016, meldonium produced a surprisingly high number of adverse analytical findings in doping control analysis, and in many cases, the athlete claimed that the substance was taken before the time of the ban. At this time point, the minimum required performance level for meldonium as a non-threshold substance was 20 ng/mL urine, based on WADAs technical docu-

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ments [6]. The high number of adverse analytical findings, however, raised the question if there is sufficient knowledge on meldonium's elimination in humans for an optimal interpretation of low urine concentrations. As a consequence, WADA gave instructions not to report cases with urine concentrations below 1 μg/mL as adverse analytical findings [7]. Today, meldonium should only be reported above a level of 100 ng/mL urine [8].

Pharmacokinetic data for meldonium in humans are limited to a few studies and previously reported data are not overall consistent. Available studies suggest both nonlinear pharmacokinetics, as well as linear pharmacokinetic properties. Furthermore, there are considerable variations in the reported half-life of meldonium, both in single and multiple dose studies.

For single dose oral administration, Zhang et al. [9] reported proportionality between dose levels (250–1500 mg) and the area under the curve (AUC), as well as proportionality in maximum plasma concentrations (C_{max}) to the administered dose. The half-life, on the other hand, was dose dependent and ranged between 3.6 and 6.6 h. Furthermore, a multiple oral dose study was conducted, in which volunteers received 500 mg meldonium three times a day for 13 days. Also here, non-linearities, in addition to accumulation, were observed. The half-life reported was 14 ± 2 h. In a different study, Peng et al [10] investigated the meldonium pharmacokinetics for 24 h after single intravenous administrations of 250, 500 and 100 mg meldonium. In contrast to Zhang et al., they reported linear pharmacokinetics with a half-life ranging from 5.6 to 6.6 h. They also monitored pharmacokinetic parameters after multiple intravenous administrations of 500 mg for 6 days, and like Zhang et al, the observed half-life was longer (15 ± 3 h) compared to single-dose administration. Hence, the authors suggested accumulation of the compound. In a further study, Cai et al. [11] carried out analysis of meldonium in plasma – and urine samples subsequent to intravenous administration of 250, 500 and 750 mg of meldonium. The estimated elimination half-life increased with increased administered dose (reported half-lives were 2.7–5.2 h), and consequently, the authors concluded that meldonium may exert non-linear pharmacokinetics in humans. Pidpruzhnykov et al. [12] investigated pharmacokinetic properties in a bioequivalence study. Plasma concentrations were monitored 24 h after single oral administrations of two generic formulations of the drug, and the pharmacokinetic elimination profile was reported to be linear (half-life = 3.6–3.7 h). They also observed a double peak in the plasma concentration-time profile, which they suggested could be associated with two areas of absorption in the gastrointestinal tract.

In the perspective of doping control analysis, one major limitation of the studies above is the relatively short sample collection time subsequent to drug administration. A few available studies, though, deal with longer sample collection times. In 2011, Liepinsh et al. [13,14] conducted a long-term study, in which they monitored plasma, as well as urine concentrations of meldonium. Volunteers were treated orally with 500 mg meldonium twice a day for a period of 4 weeks and samples were collected weekly. In addition to the treatment period, samples were collected during a washout period of 4 weeks after the end of treatment. After the 4 week wash-out period, meldonium was still present in both plasma and urine samples, and the authors concluded that meldonium accumulates after long-term treatment, and that the elimination time is treatment – and dose depended. In a recent investigation, Görgens et al. [15] examined urinary excretion profiles single-dose and multiple-dose oral application. They found that the elimination of meldonium was characterized by two phases, in which the second phase is considerably slower than the first one. The urinary detection windows expanded as much as 64 and 117 days, after single – and multiple dose administration, respectively. A further study performed by Tretzel et al. [16], where meldonium was analysed in dried blood spots, supports the presence of more than one elimination phase.

In recent years, a number of assays have been published on the analysis of meldonium in human plasma and urine [11,12,17–21]. Taking into account the polar characteristics of meldonium, a majority of the methods are based on hydrophilic interaction liquid chromatography – tandem mass spectrometry (HILIC-MS/MS) [11,12,17–19].

In this study, for the first time, the excretion of meldonium after multiple parenteral administration in six healthy volunteers was investigated. Urine samples were collected over a time period of eight months, in order to characterize the long-term excretion pattern of the drug.

2. Experimental

2.1. Design of the clinical study

Six volunteers (three male and three female) aged from 37 to 55 received three parenteral doses of Mildronates® (500 mg meldonium/5 mL), Grindex, Latvia. All volunteers received 250 mg of meldonium in the morning on day 1 and the injection was repeated on day 3 and 5. All urine samples were collected for nine consecutive days after the first administration. For the next five days, urine samples were collected once (in the morning) every day. Furthermore, one urine sample was collected once a week for the next eight months. All participants were non-athletes and on normal diets. They were healthy, not taking any diuretic medications, which could interfere with the excretion of meldonium [22].

The study was conducted in accordance with the International Conference on Harmonization guidelines for Good Clinical Practice, and was compliant with the ethical principles described in the current version of the Declaration of Helsinki. Prior to study initiation, all protocols were approved by the local ethics committee of the Sports Medicine Association of Serbia (Belgrade, Serbia).

2.2. Chemical and reagents

Meldonium dihydrate was purchased from Sigma (St Louis, MO, USA) and meldonium-d3 (internal standard) was provided by TLC Pharmachem (Ontario, Canada). Methanol of HPLC grade was obtained from Chem Lab (Zedelgan, Belgium) and water of HPLC grade was provided by Merck (Darmstadt, Germany). Formic acid (99%, ULC/MS-CC/SFC) was purchased from Bisolve (Valkenswaard, The Netherlands).

2.3. Sample preparation

To an aliquot of 100 μL urine, 50 μL of a meldonium d3 internal standard solution (1 μg mL^{-1}) and 300 μL of solvent were added. The solvent was a mixture of methanol and water in the ratio 90:10 (v/v) with 0.1% of formic acid. The sample was mixed and an aliquot of 10 μL was injected into the LC/MS/MS instrument. Samples with a meldonium concentration exceeding the highest point of the calibration curve were diluted to fall within the calibration range.

2.4. Liquid chromatography – mass spectrometry

The samples were analyzed using a CTC HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland) and an Aria Transcend TLX-1 LC system (Thermo, Austin, TX, USA) interfaced to a TSQ Vantage triple quadrupole (Thermo, Austin, TX, USA). A silica precolumn (4.0×2.0 mm, particle size 5 μm) (Phenomenex, Aschaffenburg, Germany) was used for sample clean-up and the analytical HPLC column was an Atlantis HILIC Silica (50 mm × 4.6 mm, 3 μm particle size) (Waters, Milford, MA, USA). Column selection was performed by a Maylab Mistraswitch column selector (6 column selection system) (Maylab Analytical Instruments, Vienna, Austria). Mobile