Analyses of Meldonium (Mildronate) from Blood, Dried Blood Spots (DBS), and Urine Suggest Drug Incorporation into Erythrocytes

Initially developed in the late 1970s for veterinary applications due to proposed growth-promoting effects in animals [5], meldonium has become an approved drug in selected Eastern European countries and is the subject of ongoing clinical trials focusing on the compound’s anti-ischemic and cardioprotective properties [2,3,12,15] as well as potential applications regarding diabetes, neurodegenerative disorders, and bronchopulmonary diseases. In the context of athletic performance, beneficial effects on the individuals’ physical working capacity, increased endurance performance, and accelerated recovery after physical activity were discussed [4,10,11], mentioning oral doses of meldonium of up to 2.0 g per day over 2–3 weeks in the course of pre-competition preparation phases [4]. In 2015, the World Anti-Doping Agency (WADA) initiated a one-year monitoring program [22] regarding the prevalence of meldonium (mildronate) in doping controls. Obtained data demonstrated a considerable extent of meldonium use by athletes [8,16], which was further corroborated by a significant number of declarations of anti-doping Agency (WADA) initiated a one-year monitoring phases [4]. In 2015, the World Anti-Doping Agency (WADA) initiated a one-year monitoring program [22] regarding the prevalence of meldonium (mildronate) in doping controls. Obtained data demonstrated a considerable extent of meldonium use by athletes [8,16], which was further corroborated by a significant number of declarations of anti-doping Agency (WADA) initiated a one-year monitoring program [22] regarding the prevalence of meldonium (mildronate) in doping controls. Obtained data demonstrated a considerable extent of meldonium use by athletes [8,16], which was further corroborated by a significant number of declarations of anti-doping Agency (WADA) initiated a one-year monitoring program [22] regarding the prevalence of meldonium (mildronate) in doping controls. Obtained data demonstrated a considerable extent of meldonium use by athletes [8,16], which was further corroborated by a significant number of declarations of anti-doping Agency (WADA) initiated a one-year monitoring program [22] regarding the prevalence of meldonium (mildronate) in doping controls. Obtained data demonstrated a considerable extent of meldonium use by athletes [8,16], which was further corroborated by a significant number of declarations of
observed with ca. 2.8 µg/mL 2 h post-administration, followed by a rapid decrease to ca. 130 ng/mL within the first 12 h. Although this suggested a rather rapid elimination of meldonium, the analyses of DBS samples collected subsequently at 24 h, 9 and 16 days, still yielded concentrations of ca. 80 ng/mL as illustrated in Fig. 1. This translated into an elimination profile of meldonium in urine (not specific gravity-adjusted) as shown in Fig. 2, which continued with urine samples until day 49 post-application. Peak urinary meldonium concentrations were observed 5 h post-administration with ca. 260 µg/mL, and urine samples still contained ca. 10–60 ng/mL of the drug in the pilot study test days 18–49 (432–1 176 h). Noteworthy, within the first 24 h, ca. 190 mg (38%) of the administered meldonium were excreted, while only ca. 1.5 mg were renally eliminated during the period from 24 to 48 h. The concentration profile of urine samples obtained from the multi-dose study, for which pre-, inter-, and post-administration samples up to 33 days were available (Fig. 3), was in accordance with that of the singledose administration study results. While maximum urinary meldonium concentrations of more than 1.5 mg/mL were observed during the drug administration period, urine concentrations rapidly decreased in a first elimination phase after cessation of the drug from ca. 960 µg/mL to ca. 9 µg/mL within 72 h. Subsequently, meldonium was detected at concentrations between ca. 1 and 9 µg/mL until day 33, corroborating the aforementioned slow excretion process of the drug.

This atypical elimination behavior of meldonium suggested an incorporation of the substance into the cellular fraction of blood, which was further investigated by means of whole blood samples collected from the multi-dose administration study on day 4 and day 28. Here, plasma was separated from the RBC fraction, and dried plasma spots (DPS) as well as dried spots of washed RBC fraction (20 µL each) were subjected to the same analytical protocol as the DBS. The analyses revealed that meldonium was, despite intense washing of the intact erythrocytes, still abundantly present in the RBC fraction with ca. 1 800 ng/mL on day 28. Conversely, a ca. 30-fold lower concentration was found in the corresponding plasma spot, which corroborated the hypothesis that meldonium can be incorporated into RBCs. To date, the reversibility of the suggested incorporation and underlying (active or passive) transport mechanisms are unknown and further investigations are deemed warranted, also in consideration of data suggesting a slow but distinct partitioning of the structurally similar l-carnitine between plasma and RBCs [1, 6] as well as muscle cells [14, 17, 19]. The successive and slow release of erythrocyte-entrapped meldonium into the circulation would plausibly explain the observed and beforehand unexpected detection window for meldonium, particularly in the light of an erythrocyte lifespan of approximately 70–140 days [7].

According to literature data, the estimated half-life ($t_{1/2}$) of meldonium during the initial rapid elimination phase is 5–15 h [13, 25]. The results of this pilot study suggest the existence of a subsequent second and substantially slower elimination phase, attributed to a proposed incorporation of meldonium into erythrocytes. The administration of a single oral dose of meldonium was detected in human urine for up to 49 days using established doping control analytical approaches and in DBS for at least 16 days. In consideration of the multi-dose administration data and reported accumulation effects regarding meldonium [25] and the aforementioned results that suggest the incorporation of the drug into erythrocytes allowing for sustained liberation during eryptosis, detection windows after long-term administration of
high but yet therapeutic amounts of meldonium span over several weeks and might even extend to months.

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