Monocyte Derived Macrophages as an Alternative Cell Model for the Functional Diagnosis of Creatine Transporter Deficiency

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Background

Measurement of creatine transporter activity is essential for establishing the diagnosis of creatine transporter deficiency (CTD) and to determine whether there is residual activity that might affect therapeutic responses. Primary skin fibroblasts are currently considered the gold standard cell type for functional assays. However, the minimally invasive nature of skin biopsy and the prolonged time required for fibroblast isolation and expansion limit their practical accessibility. To overcome these limitations, we investigated peripheral blood–derived cells as an alternative cellular model for the functional diagnosis of CTD.

Methods

Cell Culture and Creatine Transport :

Monocytes were isolated from healthy donor apheresis cones using Lympholyte H density gradient centrifugation following a 1:10 dilution in PBS. Peripheral blood mononuclear cells (PBMCs) were collected at the interface and washed twice in PBS. Cells were resuspended in RPMI supplemented with 10% endotoxin-free fetal bovine serum (FBS) and seeded on appropriate plasticware. After 30 min incubation at 37 °C and 5% CO_2, nonadherent cells were removed by three washes with prewarmed EBSS. Adherent monocytes were used immediately or differentiated into monocyte-derived macrophages (MDMs) by culture in complete medium containing 50 ng/mL recombinant human GM-CSF for up to 5 days. On the day of the experiment, cells were washed twice with Earle's balanced salt solution containing 5.5 mM D-glucose and incubated for 1 h at 37 °C in the presence of indicated concentrations of d5-creatine. Transport was terminated by three rapid washes with ice-cold 0.3 M urea. Non-saturable transport was determined in the presence of 2 mM creatine. Intracellular creatine was extracted with 0.3 mL ice-cold ethanol, followed by addition of d3-creatine (400 nM) as an internal standard. Results were normalized to protein content and expressed as pmol/mg protein/hour. Saturable transport was calculated as the difference between total and non-saturable uptake.

Quantitative RT-PCR

Total RNA was extracted from adherent monocytes and MDMs using a Miniprep Kit. For monocytes, RNA was isolated from combined adherent and nonadherent cells. One microgram of RNA was reverse transcribed, and 40 ng cDNA was used for amplification. Expression of SLC6A8 and the housekeeping gene RPL15 was assessed using a SYBR Green assay. *SLC6A8* expression was normalized to *RPL15* and presented relative to freshly isolated monocytes (set to 1).

LC-MSMS:

d3- and d5-creatine were quantified by UPLC-MS/MS using an ACQUITY UPLC I-Class system coupled to a Xevo TQ-XS mass spectrometer. Samples were dried and derivatized with butanolic HCl prior to analysis. Separation was achieved on a reverse-phase column (Acquity UPLC BEH C18, 1.7 µm, 2.1 × 100 mm) with an in-line pre-column filter (0.2 µm), and detection was performed using tandem mass spectrometry.

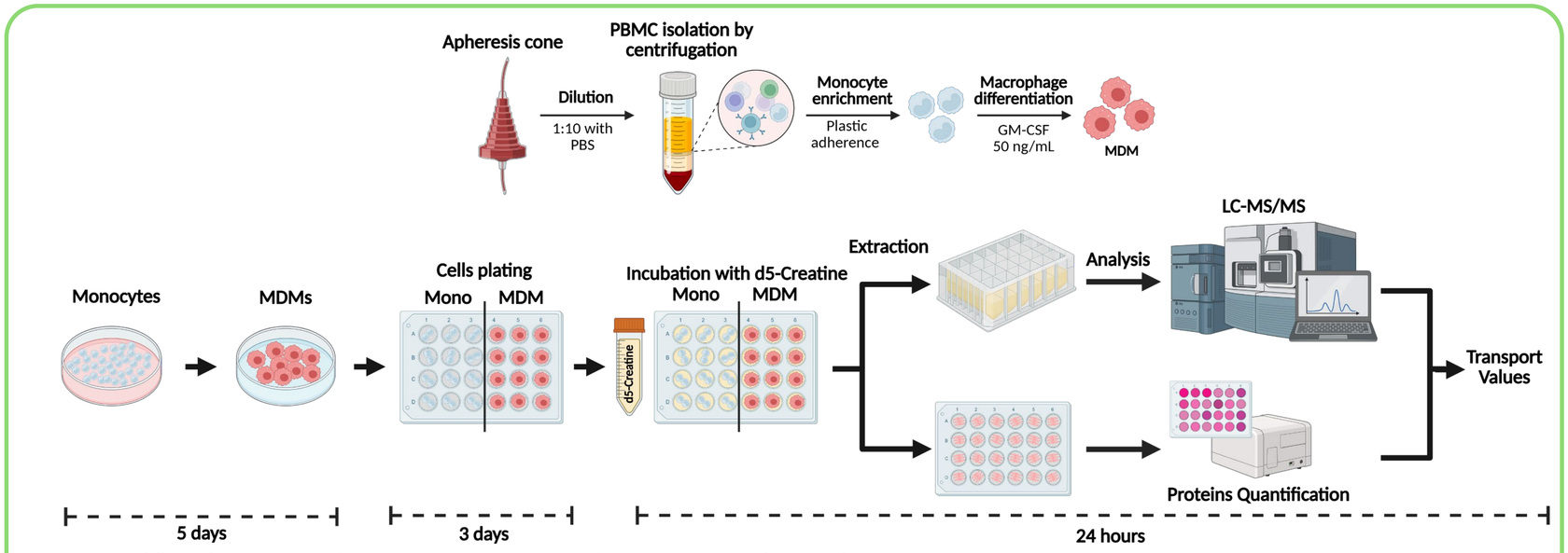

Figure 1. Workflow of monocytes isolation, conversion to MDM, and creatine transport functional test.

Results

- Monocytes exhibited minimal creatine uptake (≈29 pmol/mg protein/h), whereas MDM showed a marked increase in saturable creatine uptake (≈517 pmol/mg protein/h), comparable to that observed in primary fibroblasts (≈607 pmol/mg protein/h) (Figure A).
- Consistent with these findings, SLC6A8 mRNA expression was increased approximately ninefold in MDM compared with monocytes (Figure B).
- Kinetic analysis demonstrated that creatine uptake in MDM was linear for up to two hours (data not shown) and exhibited a Km of 44.7 ± 8.2 µM, closely matching fibroblast kinetic properties (Figure C).

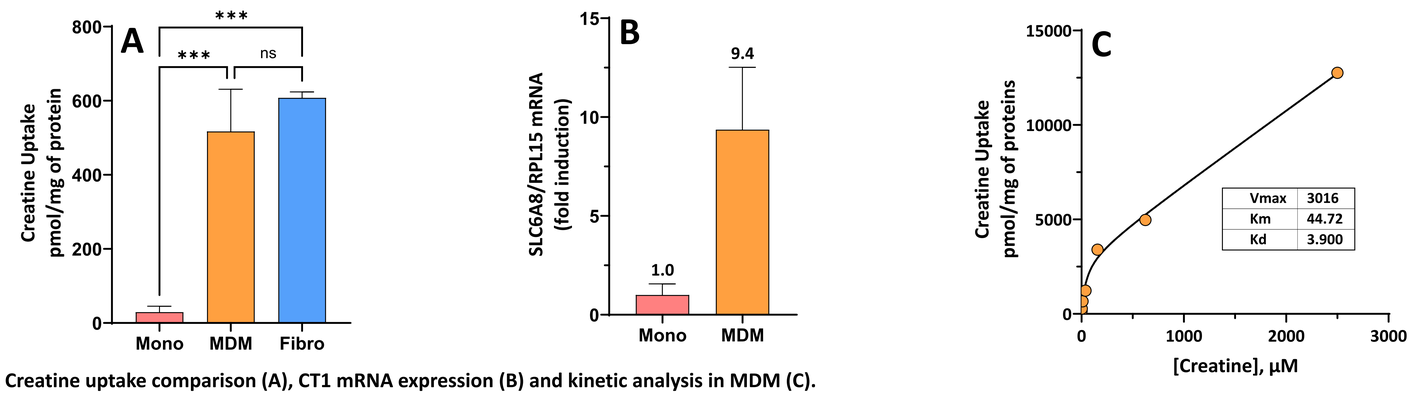

Figure 2. Creatine uptake comparison (A), CT1 mRNA expression (B) and kinetic analysis in MDM (C).

Creatine transport was measured for 1 h in human monocytes, MDM, and fibroblasts in the presence of 20 µM d5-creatine. Points are averages of triplicates ± SD; ***P<0.001 (A). Expression of CT1 transporters was determined in freshly isolated monocytes and MDM by quantitative RT-PCR; data are expressed as fold induction. Points are averages of triplicates ± SD (B). MDM were incubated in the presence of d5-creatine at the indicated concentrations (0.1-2500 µM) for 1h (C).

Conclusion

Monocyte derived macrophages represent a promising, faster, and less invasive cell system as compared to skin fibroblasts for the functional diagnosis of CTD.

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