Amiloride is a suitable fluorescent substrate for the study of the drug transporter human multidrug and toxin extrusion 1 (MATE1)

Abstract

Human multidrug and toxin extrusion 1 (MATE1; SLC47A1) is highly expressed in the kidneys and the liver. It plays a significant role in drug and endogenous compound disposition, and therefore, a rapid evaluation of its inhibition is important for drug development and for the understanding of renal and hepatic physiology. Amiloride is a potassium-sparing diuretic used for treating hypertension; it also demonstrates strong fluorescence in organic solvent or detergent solutions. In this study, we investigated the transport characteristics of amiloride by human MATE1. Cellular accumulation of amiloride was evaluated in control vector- or MATE1-transfected HEK293 cells. Cells were lysed with 1% sodium dodecyl sulfate, and fluorescence was measured using a microplate reader at wavelengths of 364ex and 409em. With ammonium prepulse-induced intracellular acidification, MATE1 transported amiloride at an extracellular pH of 7.4. The uptake demonstrated an overshoot phenomenon and saturated, with the Km and Vmax being 23.5 μM and 1.01 nmol/mg/min, respectively. MATE1-mediated amiloride transport also presented with a bell-shaped pH profile that reached a maximum pH value of 7.4. The inhibitor sensitivity of MATE1-facilitated amiloride transport was similar to those of known substrates, such as tetraethylammonium and metformin. Among the tested inhibitors, pyrimethamine demonstrated the most potent inhibition with an IC50 value of 0.266 μM. Furthermore, MATE1 was found to be inhibited by fampridine, which was previously considered to be a non-inhibitor of MATE1. This study demonstrates that amiloride is a suitable fluorescent substrate for the in vitro study of the transport activity of MATE1.