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The role of monocarboxylate transporters in uptake of lactic acid in HeLa cells

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Abstract

This study was aimed to identify the monocarboxylate transporters (MCTs) in HeLa cells and to delineate their role in transportation of L-lactic acid. The functional role of MCTs in lactic acid transport was evaluated at various mucosal pHs (4.5–7.4) or in the presence of various loading doses (0.2–2 mM) of lactic acid, MCT substrates (nicotinic acid, *n*-butyric acid, etc.) and inhibitors (α-cyano-4-hydroxycinnamate and *para*-chloromercuribenzoic acid). The molecular properties of MCTs were characterized using reverse transcription-polymerase chain reaction (RT-PCR). The uptake rate of lactic acid by HeLa cells significantly increased from 0.353 ± 0.052 to 1.103 ± 0.196 μmol/mg protein as the extracellular pH changed from 7.4 to 4.5, indicating that activities of MCT were mediated through H^+-linked mechanism. The uptake profile of lactic acid followed the saturable process with the K_m value of 0.53 mM. The uptake rate of lactic acid is concentration dependent and is reduced in the presence of MCT inhibitors. MCT isoforms 1, 5 and 6 in HeLa cells were identified by RT-PCR. HeLa cell line can be used as an effective screening tool for intravaginally administered drugs targeted toward MCT.

Keywords: Human cervical cells; Monocarboxylate transporters; Lactic acid; Vaginal homeostasis; RT-PCR

1. Introduction

Halestrap and Denton (1974) initially demonstrated a saturable influx of monocarboxylates into mitochondria, which could be inhibited by the addition of α-cyano-4-hydroxycinnamic acid (α-CHC), a potential inhibitor for the monocarboxylate transporter. Monocarboxylate transporters (MCTs) in normal cells play an important role in regulating the influx and efflux of monocarboxylate compounds, such as L-lactic acid, acetic acid and pyruvic acid. This transport process is known to be facilitated by the concentration gradients of lactate and protons (Palmieri et al., 1996; Price et al., 1998; Majumdar et al., 2005).

Subsequent functional studies have revealed the presence of the proton-coupled monocarboxylate transporter (MCT) in the plasma membrane of various cell types. To date, the characteristics of MCTs have been extensively studied in cell types, such as erythrocytes (Halestrap, 1976), chondrocytes (Meredith et al., 2002), hepatocytes (Jackson and Halestrap, 1996), tumor cells (Carpenter and Halestrap, 1994), cardiac myocytes (Wang et al., 1994), skeletal-muscle cells (Juel, 1997), myocytes and neutrophils (Meredith et al., 2002; Hertz and Dienel, 2005) and brain astrocytes (Broer et al., 1997), but not in cervical or vaginal cells. Subsequently, identification and characterization of MCTs in the vaginal epithelium are essential in screening and classifying pharmaceuticals aimed to be delivered via intravaginal route.

The regulation of lactic acid formation and its transport through the vaginal epithelium have been crucial for maintaining vaginal homeostasis (Melis et al., 2000). One of the major defense mechanisms of vagina against exogenous microbes comprised the normal microbial flora, predominantly lactobacilli, which drove out exogenous microbes by maintaining a low pH and generating organic acids, such as lactic acid, peroxide and bacteriocins or lactocins (Aroutcheva et al., 2001; Wiberg-Itzel et al., 2005). The organic acids maintain the vaginal pH at <4.5, thereby creating an inhospitable environment for the growth of pathogens, and acidic vaginal pH is detrimental for the survival of microbes and sperms (Chien and Lee, 2002; Choe et al., 2004).

Since cervix protrudes into the uppermost part of the vagina and is covered by the same type of cells as the vaginal lining,

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HeLa, a well-established and easily-available human cervical adenocarcinoma cell line, has been widely used for screening of chemotherapeutic agents applied in vagina and cervix (Shiraishi et al., 2005). Even though HeLa cell line is derived from adenocarcinomas, various study showed its usefulness in elucidating the mechanism involved with biochemical activities including the estrogen receptor subtypes ERalpha and ERbeta (Papoutsi et al., 2005) and mitochondria and mitochondria cytochrome *c* (Wang et al., 2005).

In this study, it is hypothesized that the proton linked monocarboxylate transporters which facilitate the uptake process of exogenous compounds having a monocarboxyl group are also present in the vaginal epithelium. We used L-lactic acid as a model drug and HeLa cells as a model cell line. Since the pK_a of lactic acid is 3.86 and thus, it dissociates into its ionic form in vaginal cavity (pH 4.5) (Halestrap and Price, 1999), plasma membrane is passively impermeable to the lactate and H^+ species. There should be a specific carrier system for lactic acid to transport across the membrane. The functional role of MCTs in lactic acid transport was evaluated by examining its uptake rates at various mucosal pHs (4.5–7.4) or in the presence of various loading doses (0.2–2 mM) of lactic acid, MCT substrates and inhibitors in HeLa cells. The molecular level characterization of MCT was performed by reverse transcription polymerase chain reaction (RT-PCR). The physiological function of the MCTs may be utilized for targeting drugs to systemic application following intravaginal administration.

2. Materials and methods

2.1. Materials

HeLa cell lines and growth media were purchased from American Type culture collection (ATCC, Manassas, VA). ^{14}C L-lactic acid (131 mCi/mmol) was purchased from American radiolabeled chemicals (St. Louis, MO). Hanks balanced salt solution (composition: 137 mM NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4$, 1.2 mM $CaCl_2$, 4.2 mM $NaHCO_3$, 0.33 mM Na_2HPO_4, 0.4 mM K_2HPO_4), Trizol reagent and specified primers for MCT were purchased from invitrogen (Carlsbad, CA). Reverse transcription kit and PCR II core system were purchased from Promega (Madison, WI). All other reagents and buffers were of reagent grade and purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Cell culture

HeLa cell line was maintained in Eagles modified minimum essential medium (MEM) containing 10% fetal bovine serum and 2% penicillin/streptomycin solution. The cells were grown in 75 cm^2 tissue culture flasks at 37 °C in a 5% CO_2 incubator.

2.3. Effects of extra cellular pH on the uptake rate of lactic acid in HeLa cells

To evaluate the role of MCTs in the transport of lactic acid through the vaginal epithelium, the effects of pH on its uptake was evaluated using HeLa cells. About 2.5×10^6 HeLa cells were seeded on 12 well plates. Once cells were confluent, the cells were rinsed with 25 mM *N*-(2-hydroxyethyl) piperazine-*N*-2-ethanesulfonic acid (Hepes)-buffered Hank's balanced salt solution (HHBSS, pH 7.4) for 30 min. A 1 mM L-lactic acid solution spiked with ^{14}C L-lactic acid at a concentration of 0.5 μCi/mL or 0.007 μmol (original solution – 131 mCi/mmol) was added to the cells and incubated in the buffer solutions (pHs of 7.5, 6.5, 5.5 and 4.5) for 1 min at 37 °C. The uptake process was terminated by rinsing the cell monolayer with ice-cold HHBSS (pH 7.4). After lysing the cells with the Triton X cell lysis solution, the amount of lactic acid uptake in the cells was determined by analyzing radio labeled L-lactic acid using a scintillation counter (Beckman Coulter, Fullerton, CA).

The concentration (in μmol) of the sample and the dosing solution is calculated according to the disintegrations per minute (DPM). The protein content in each well was measured by the BioRad assay method. Bovine gamma globulin was used as a standard (Tarpey et al., 1995). The uptake rate of lactic acid was expressed as μmol/mg protein/min.

2.4. Effects of inwardly directed H^+-gradient on the uptake rate of lactic acid in HeLa cells

The effect of an inwardly directed H^+-gradient on the uptake rate of lactic acid was investigated. ATP-depleted cells were used to demonstrate that the energy source for MCT isoforms was inwardly directed H^+-gradient rather than ATP. ATP depleted cells were prepared by pretreating the cells with 10 mM sodium azide (AZ) plus 10 mM de-oxy glucose (DOG) in glucose-free HHBSS (pH 7.4) for 20 min (Nagasawa et al., 2002). The reaction was initiated by the addition of 1 mM L-lactic acid spiked with ^{14}C L-lactic acid in 25 mM 4-morpholineethanesulfonic acid-Hanks balanced solution (MES-HBSS, pH 4.5) or 25 mM HHBSS (pH 7.4) to the wells, being followed by incubation for 30 min at 37 °C. The amount of lactic acid uptake in ATP-depleted cells was measured at various time points.

2.5. Effects of proton Ionophores on the uptake rate of lactic acid

The effects of carbonyl cyanide-*p*-trifluoromethoxy phenyl hydrazone (FCCP), a proton ionophore, on the uptake rate of lactic acid were evaluated in ATP-depleted cells to determine whether lactic acid uptake is pH-dependent. ATP depleted cells at pH 4.5 were treated with FCCP (10 μg/mL) and incubated with 1 mM L-lactic acid spiked with ^{14}C L-lactic acid for 1 min at 37 °C. The uptake rate of lactic acid was determined and compared with that of the control (FCCP non-treated).

2.6. Effect of the loading dose of lactic acid on its uptake in HeLa cells

To characterize the carrier-mediated transport of lactic acid in HeLa cells, the effect of various loading doses of lactic acid on its uptake rate was investigated. After rinsing the cell monolayer