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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

Table 1
Forward and reverse oligonucleotide primer sequences used for RT-PCR screening of monocarboxylate (MCT) isoforms in human cervical adenocarcinoma cells

MCT isoforms	Oligonucleotide sequence	
	Forward	Reverse
MCT-1	TCTACACTTAAAATGCCA CCAGCAGTTGGAGGTCC	AAAAACACCAAACACACA CATATCTCACACATAGCAC
MCT-2	CTGAGGATCCCCACTAGA GGAGCAGAAATG	ATCGCTGAGACTAGATGT GATTCTTGT
MCT-3	TATTTTACAAACTGGACTG GCTCAGGCAG	GGTTTATTTCCATCTTCCAG GGGTCCAAA
MCT-4	CGCCTTGTCCTCCAAAGTG CTGGGAT	TTTTGGAGGGATCATATACC ATTCCAGT
MCT-5	ATCTCAGCTGCCCTCTTCAT GGGTGGCAGCTT	TCAAGGCTCAGAGGTGGTCA ACAATTTGGCC
MCT-6	GTTCACGTGCAAATGGAGC CGGTATGA	AACCACTTTGCCAGTTCATG TCACT

with HHBSS at pH 7.4 for 30 min, the cells were incubated with various concentrations of L-lactic acid (0.2–2 mM) spiked with ^{14}C L-lactic acid in 25 mM MES-HBSS at pH 4.5 for 1 min. The amounts of lactic acid uptake by HeLa cells were determined by analyzing radio labeled L-lactic acid using a scintillation counter.

2.7. Effects of MCT substrates/inhibitors on the uptake rate of lactic acid

To evaluate the competitive inhibition of the uptake rate of lactic acid in HeLa cells, the uptake rate of ^{14}C L-lactic acid was examined in the presence of other monocarboxylic acid substrates, such as *n*-butyric acid and nicotinic acid, or inhibitors, such as α-cyano-4-hydroxycinnamic acid (αCHC) and *para*-chloromercuribenzoicacid (pCMBA). Briefly, confluent cells in 12 well plates were rinsed with HHBSS for 30 min and the cells were incubated with the solutions containing butyric acid (1–20 mM), nicotinic acid (1–20 mM), α-CHC (0.2–1) or pCMBA (0.2–1) for 15 min. The pH of all solutions was maintained at 4.5. The cells were then incubated with 1 mM L-lactic acid spiked with ^{14}C L-lactic acid in MES-HBSS (pH 4.5) in the presence of aforementioned inhibitors for 1 min at 37 °C. The amount of lactic acid uptake was determined using the method as described earlier.

2.8. RNA extraction and RT-PCR study

To identify isoforms of MCT in HeLa cells, reverse transcription-polymerase chain reaction (RT-PCR) was conducted. About 3 million HeLa cells were seeded on a Petri dish and the growth medium was changed every 2 days. Once the cells were confluent, RNA was extracted according to the procedure previously reported (Chomczynski and Sacchi, 1987; Hosoya et al., 2001). Briefly, the growth medium was replaced with 800 μL of Trizol cell lysis reagent (Invitrogen, Carlsbad, CA). The RNA pellet was extracted by sequential addition of phenol, $CHCl_3$ and isopropanol. The samples were centrifuged at 12,500 rpm.

RNA, extracted from HeLa cells, was analyzed for MCT isoforms by reverse transcription-polymerase chain reaction (RT-PCR) using Reverse Transcription Kit (Promega Inc., Madison, WI) and synthetic oligonucleotides (Table 1) for MCT isoforms (Hadjiapiou et al., 2000). Polymerase chain reaction was run for 35 cycles with denaturation for 1 cycle at 95 °C for 2 min, annealing at 56 °C for 1 min, extension at 72 °C for 1 min for 35 cycles, and final extension for 1 cycle at 72 °C for 5 min in a DNA Thermal cycler (Midwest Scientific, St. Louis, MO). The polymerase chain reaction products were analyzed on 3% agarose gel.

2.9. Statistical analyses

The reaults were expressed as mean ± S.E. Comparisons between groups were performed by analysis of variance (ANOVA). A difference with a P value of 0.05 or less was considered to be statistically significant.

3. Results

3.1. Effects of extra cellular pH on the uptake rate of lactic acid

The effects of extracellular pH on the uptake rate of lactic acid by HeLa cells are shown in Fig. 1. The rate of lactic acid uptake at the apical surface significantly decreased as extracellular pH changed from 4.5 to 7.4. The uptake amounts of lactic acid were 0.353 ± 0.052 and 1.103 ± 0.196 μmol/mg protein/min at pH 7.4 and 4.5, respectively. The uptake profile of lactic acid at the low pH marked the higher uptake rate of L-acetic acid, revealing that there are proton coupled (i.e., H^+ ion-dependent) mechanisms behind the uptake rate of lactic acid by HeLa cells. Based on the results of this study, we conducted the subsequent

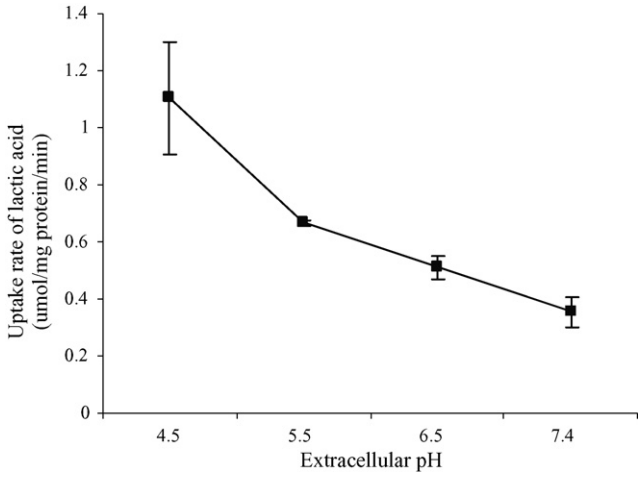

Fig. 1. Effects of the extra cellular pH on the uptake rate of lactic acid in HeLa cells. Each point represents the mean ± standard deviation of the mean (n = 3–4).

experiments with the cell growth medium whose pH was set at 4.5, at which the maximum uptake rate of lactic acid was observed.

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

To determine whether or not the uptake rate of lactic acid by cervical cells are affected by an inwardly directed H^+-gradient, the profiles of lactic acid uptake by ATP-depleted HeLa cells were examined as a function of time. It was previously shown that the pretreatment of cells with 10 mM AZ plus 10 mM DOG in glucose-free medium caused a decrease in ATP by about 80% (Nagasawa et al., 2002). As shown in Fig. 2, the uptake rate of lactic acid for 30 min in the presence of the higher H^+-gradient (pH 4.5) was significantly greater than that in the presence of the lower gradient (pH 7.4). Based on the results of this study, a time point of 1 min which lies on the linear part of the uptake versus time profile was chosen as the optimum experimental time for investigation of lactic acid uptake profiles in the subsequent experiments.

The treatment of the ATP-depleted cells with FCCP (carbonyl cyanide *p*-trifluoro-methoxyphenylhydrazone), a proton ionophore, significantly decreased the uptake rate of lactic acid from 0.997 ± 0.056 to 0.639 ± 0.146 μmol/mg protein/min, indicating that the uptake process of lactic acid was accelerated by an inwardly directed H^+-gradient. The results of this study demonstrated that proton-linked transporters present in HeLa cells are involved in the transport of lactic acid.

3.3. Effects of the loading dose of lactic acid on its uptake by HeLa cells

To characterize the carrier-mediated transport of lactic acid in HeLa cells, the effect of the loading dose of lactic acid on its uptake rate was investigated. As shown in Fig. 3, the amount of lactic acid uptake on the apical surface at a loading concentration of 0.2 mM was 0.527 ± 0.066 μmol/mg protein/min as compared to 1.953 ± 0.408 μmol/mg protein/min at a loading concentration of 2 mM. The viability of HeLa cells as evaluated by the trypan blue exclusion method remained above 90%, which is consistent with the previous studies (Walum et al., 1990). The results of this study showed that the uptake rate of lactic acid by HeLa cells is loading concentration dependent.

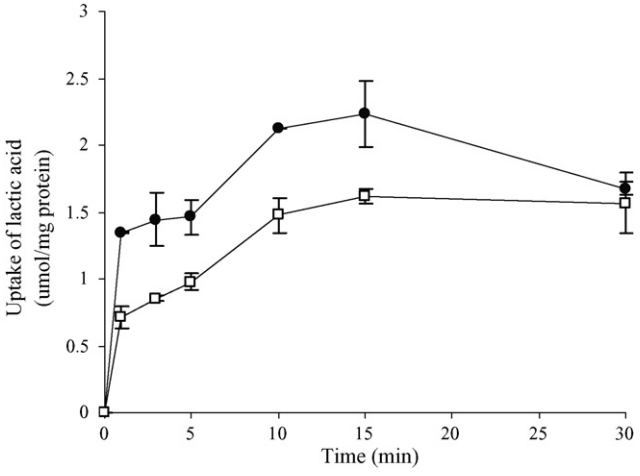

Fig. 2. Effects of the inwardly directed H^+-gradient on the uptake rate of lactic acid in HeLa cells. ATP-depleted cells were incubated with 1 mM L-lactic acid in MES-HBSS at pH 4.5 (closed circles) or HHBSS at pH 7.4 (open squares) for the indicated times at 37 °C. Each point represents the mean ± standard deviation of the mean ($n = 3$–4).

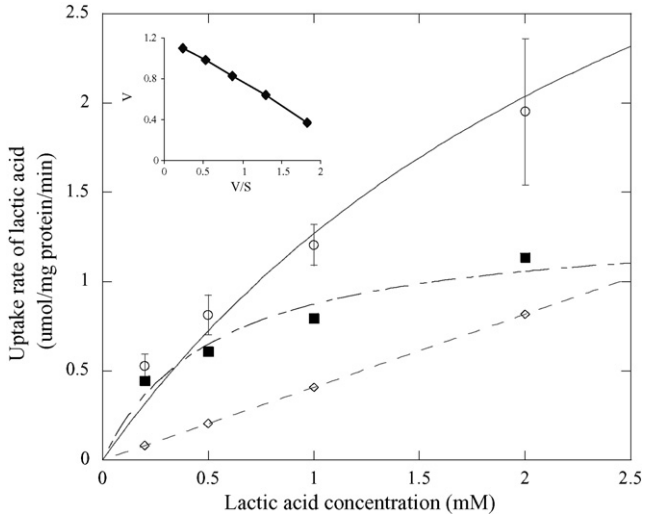

Fig. 3. Effects of the loading doses of lactic acid on its uptake rate in HeLa cells. The darker line represents the total uptake of L-lactic acid. Each point represents the mean ± standard deviation of the mean ($n = 3$–4). The upper dotted line represents the uptake for the saturable component calculated from the kinetic parameters obtained as described in the text. The lower dotted line represents the uptake for the nonsaturable component calculated from the kinetic parameters. Inset shows Eadie–Hofstee plot (V vs. V/S) for the effect of loading dose of lactic acid on its uptake rate in HeLa cells.

The kinetic evaluation of the uptake profile of lactic acid was performed using the Michaelis–Menten equation, $V = ((V_{max}S)/(K_m + S)) + K_dS$. The uptake profile of lactic acid followed Michaelis–Menten kinetics with K_m and V_{max} of 0.53 mM and 1.34 ± 0.10 μmol/mg protein/min, respectively. The estimated total uptake rate was differentiated into carrier mediated uptake and passive diffusion uptake using a kaleidograph approach. As shown in Fig. 3, the uptake rate of lactic acid due to passive diffusion remained constant irrespective of its concentration. The graph representing the carrier-mediated transport gradually increased as a function of lactic acid concentration, and reached a plateau at concentrations greater than 1 mM, revealing the involvement of the saturable transporters in uptake of lactic acid by HeLa cells. When these values were plotted as V versus V/S in an Eadie–Hofstee plot, a linear kinetic profile representing the activity of a major MCT isoform that is responsible for a single type of the saturable uptake process was obtained. The results of this study further confirmed the involvement of MCT transporters present in HeLa cells in the transport of lactic acid.

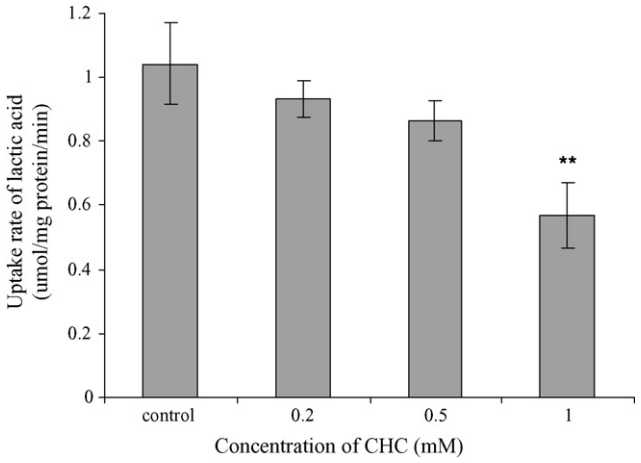

Fig. 4. Effects of α-cyanohydroxycinnamic acid (CHC) on the uptake rate of L-lactic acid in HeLa cells. Each point represents the mean ± standard deviation of the mean ($n = 3$–4). $^{**}P < 0.01$, significantly different from control.

3.4. Effects of substrates/inhibitors on the uptake rate of lactic acid

The MCT inhibitors tested in this study significantly reduced the uptake rate of lactic acid in HeLa cells (Fig. 4). The uptake rates of lactic acid in the presence of 1 mM αCHC and 1 mM *p*-CMBA were 0.568 ± 0.10 and 0.762 ± 0.031 μmol/mg protein/min, respectively, both of which were significantly lower as compared with the control (1.042 ± 0.128 μmol/mg protein/min). As shown in Fig. 5, the typical inhibition profiles of lactic acid were demonstrated in the presence of other monocarboxylates including *n*-butyric acid. The uptake rate of lactic acid was reduced by 55% in the presence of 20 mM *n*-butyric acid (0.623 ± 0.069 μmol/mg protein/min) as compared with the control (1.376 ± 0.233 μmol/mg protein/min), whereas it marked a 65% reduction in the presence 20 mM nicotinic acid (0.476 ± 0.091 μmol/mg protein/min). These data along with the results from the Michaelis–Menten estimation revealed that lactic acid was transported across the HeLa cell via MCTs, which are a single type saturable transporter.

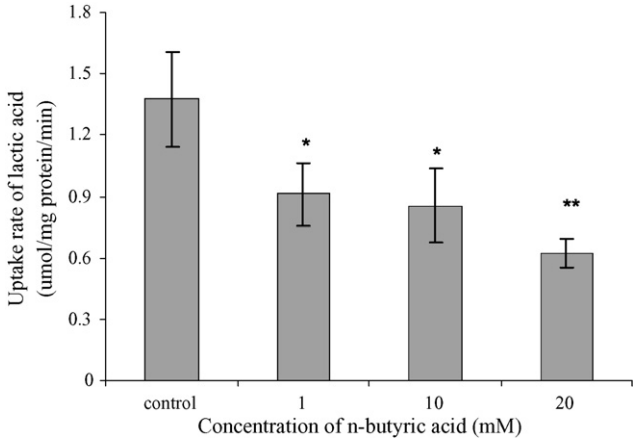

Fig. 5. Effects of *n*-butyric acid on the uptake rate of L-lactic acid in HeLa cells. Each point represents the mean ± standard deviation of the mean ($n = 3$–4). $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from control.

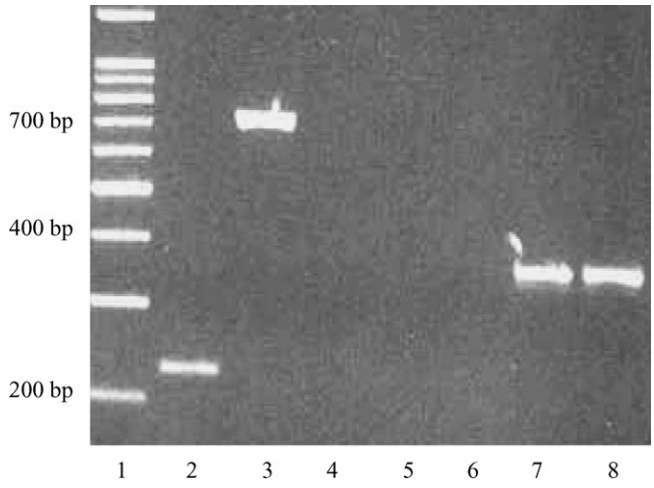

Fig. 6. RT-PCR on MCT in HeLa cells. Lane 1 represent 100 bp DNA ladder, lane 2 represent GAPDH and lanes 3–8 represent MCT 1–6, respectively. bp: base pairs.

3.5. RT-PCR study

Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed on RNA extracted from HeLa cells to identify the specific isoforms of MCT. As shown in Fig. 6, the results of the RT-PCR study with specific oligoprimers for MCT isoforms confirmed a specific band at about 700, and 2 bands at about 380 bp; which were identified as MCT-1, 5 and 6, respectively. The band positions of the RT-PCR study are similar to those in the previous report (Halestrap, 2004) and sequencing of the PCR product and BLAST search (NCBI) further confirmed that the PCR product showed maximum homology to corresponding hMCT isoforms. These results demonstrated that MCT is functionally active and MCT-1, 5 and 6 in HeLa cells are involved in the apical uptake of monocarboxylates. An investigation on which isoform is predominant in lactic acid transport is currently under study. This study demonstrated importance of establishing functional activity and biochemical expression of transporters in HeLa cell line. It was suggested that HeLa cells can be used as an effective screening tool to select a lead candidate from various intravaginally delivered endogenous/exogenous agents targeted toward MCT.

4. Discussion

The human vagina represents a potential site that offers a valuable route for drug delivery for both local and systemic applications. Even though intravaginal drug delivery is particularly appropriate for drugs associated with women's health issues, it also has applications in general drug delivery within the female population (Woolfson et al., 2000; Vermani and Garg, 2000). The vaginal route has been traditionally used for the delivery of locally active drugs, such as antibacterial, antifungal, antiprotozoal, antiviral, labor-inducing and spermicidal agents, prostaglandins and steroids, but is now gaining importance as a possible site for systemic delivery.

Drugs are transported across the vaginal membrane by three primary pathways: the transcellular route by which diffusion occurs through the cell due to a concentration gradient; the intercellular route, where diffusion occurs through spacing between the cells; or by a vesicular and receptor-mediated transport (Chien and Lee, 2002). Transporters, such as prostaglandin transporter (PGT) (Schuster, 1998; Lu et al., 1996) and Purinergic receptor (P2X5) (Groschel-Stewart et al., 1999) have been identified in the vaginal epithelium.

This study is aimed to identify and characterize the monocarboxylate transporters (MCTs) in the vagina. The transport of monocarboxylates into various mammalian cells is known to be mediated by MCTs. Numerous drugs containing a carboxyl group make themselves potential substrates for MCTs. There have been 14 MCT sequences identified in mammals (MCT 1-14), each having different tissue distribution profiles. To date, only first four MCTs (MCT 1-4) have been functionally characterized and their direct involvements of proton linked lactate and pyruvate transport have been demonstrated (Halestrap and Meredith, 2004). Studies have revealed the diversities in kinetics, substrate and inhibitor characteristics of MCT isoforms in mammalian cells and that these differences are related to the metabolic requirements of the tissue in which they are expressed (Price et al., 1998; Jackson et al., 1997; Wilson et al., 1998).

The charged form of lactic acid requires a specific transport mechanism to penetrate into the vaginal epithelium. Even though lactic acid mainly exists in its ionized form in the pH range studied (pH 4.5–7.4), the highest uptake rate was observed at pH 4.5. This can be explained by the presence of the higher number of protons at pH 4.5 that result in elevated H^+-dependent lactic acid uptake. The difference in the uptake rates of lactic acid at different pHs supported the role of proton gradient in the regulation of lactic acid in cervical cells. It was further confirmed by the significant decrease in the uptake rate of lactic acid in the presence of protonophore, FCCP, which acts as a proton carrier to dissipate the proton gradient across the apical membrane, thereby decreasing the driving force required for proton-cotransport events (Wu et al., 2000). The unionized forms of lactic acid were likely to be transported through vaginal membrane by passive diffusion to some extent, which was supported by the observation that the insignificant amount of lactic acid was taken up into the cell in the absence of proton gradient (i.e., at pH 7.4). However, the influence of proton gradient at lower pHs indicated that a proton-mediated transport system was involved in lactic acid transport across HeLa cells.

The functional role of MCTs in lactic acid transport were characterized by examining the uptake profiles of lactic acid in the presence of MCT inhibitors, such as α-cyano-4-hydroxycinnamate and *p*-chloromercuribenzoic acid. Both inhibitors significantly reduced the uptake rate of lactic acid by HeLa cells. Other monocarboxylates, such as nicotinic acid and *n*-butyric acid, also reduced the uptake rate of lactic acid, which are comparable to the previous studies on Caco-2 cells (Hadjiapiou et al., 2000; Wu et al., 2000).

The uptake profile of lactic acid in HeLa cells followed typical Michaelis–Menten kinetics with K_m and V_{max} values of 0.53 mM and 1.34 μmol/mg protein/min, respectively. The Eadie–Hofstee plot for saturable component of lactic acid uptake was linear, implying the involvement of a single major isoform of MCT in the apical uptake of lactic acid. The MCT isoforms 1, 5 and 6 were identified by RT-PCR. Although the presence of MCT-4 was detected, the band was barely visible. The difficulty of detecting all isoforms of MCT in cultured cells may contribute to the lower V_{max}. The characterization of each MCT isoform in cultured cells is further hindered by the simultaneous expression of multiple isoforms with very similar substrate specificity. As a continuous work, an investigation on determining the contribution quotient of individual MCT isoforms to lactic acid transport will be followed.

5. Conclusions

In this study, the involvement of monocarboxylate transporters in the uptake of lactic acid by HeLa cell was demonstrated and the functional role of MCTs in HeLa cells was elucidated. The MCT isoforms 1, 5 and 6 were identified in HeLa cells using the PCR analysis. Further studies are required to elucidate the role of each MCT isoform on lactic acid and other monocarboxylate drug transport across vaginal epithelium. MCTs may provide a potential means for the intravaginal delivery of monocarboxylate drugs and HeLa cell line can be used as an effective screening tool for drugs targeted toward MCT.

Acknowledgement

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