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Original

Characterization of Urate Transport System in JAR and JEG-3 Cells, Human Trophoblast-derived Cell Lines

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SUMMARY

Urate (uric acid) is the major inert end product of purine metabolism in humans. Since it is water soluble, it requires a membranous protein called transporter for its permeation across the plasma membrane. Increased blood urate level is often seen in preeclampsia, but its precise mechanism remains unknown. Syncytiotrophoblasts function as a barrier between maternal blood and fetal one so called "blood-placental barrier". So far, the expression of several urate transporters was identified in these cells, but it is still unclear about their contribution to urate handling in blood-placental barrier. In this study, we investigated the expression of urate transporters and the properties of [^{14}C] urate transport in both JAR and JEG-3, human choriocarcinoma cells as a model of human placenta. Conventional PCR analysis revealed that both JAR and JEG-3 cells express strongly breast cancer resistance protein (BCRP/*ABCG2*) mRNA. Uptake of [^{14}C] urate by these cells is time-dependent with Na^+- and Cl^--independent and voltage-insensitive manner and is not inhibited by benzbromarone, a representative renal urate transport inhibitor. Then, we focused on BCRP which shows strong mRNA expression and found that these cells have urate efflux property that is sensitive to fumitremorgin C (FMC), a BCRP inhibitor. These results suggest that BCRP is one of the important components for urate handling in human placenta in pathophysiological condition such as preeclampsia.

key words : urate, placenta, preeclampsia, hyperuricemia, transporters

INTRODUCTION

In humans, serum uric acid (urate) level is determined by the balance between hepatic production by the enzyme xanthine oxidase (XO) and renal excretion mediated by tubular urate transporters in physiological condition[1]. Thus, hyperuricemia is caused by the imbalance between production and excretion of urate. Preeclampsia is one of the pregnancy complications which is known to be closely related to the elevation of maternal urate level[2]. Although it is suggested that hyperuricemia in such patients is induced by increased placental urate production[3], the mechanism how can urate, produced in syncytiotrophoblasts, enter into the plasma membrane has not been clarified yet. Recently, Uehara *et al.* clarified that urate

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moves through the paracellular route in placenta by simple diffusion using one of the human choriocarcinoma cell lines BeWo[4], but it is still uncertain because this cell is only one kind of trophoblast-derived epithelial cancer cell lines and there are still other kind of the cells named JAR and JEG-3, normally analyzed together with BeWo.

In this study, we examined the expression of several urate transporters and characterized urate transport properties in JAR and JEG-3 cells, other human choriocarcinoma cell lines that are usually used for the analysis of organic solute transport[5] or drug-metabolizing enzyme[6] to further clarify the contribution of transporters for the permeation of urate in placenta.

MATERIALS AND METHODS

Materials

[^{14}C]Urate was purchased from Moravek Inc. (Brea, CA, USA). URAT1 inhibitor benzbromarone, BCRP inhibitor fumitremorgin C, and other chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). JAR (Catalog number : HTB-144^{TM}) and JEG-3 (Catalog number : HTB-36^{TM}), human choriocarcinoma cell lines, were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA).

Reverse transcription polymerase chain reaction (RT-PCR)

RNA samples were collected using the RNeasy Kit (Qiagen, Venlo, Netherlands) from JAR and JEG-3 cells. cDNA was synthesized with PrimeScript^{TM} RT reagent Kit with gDNA eraser for RT-PCR (Takara Bio Inc., Shiga, Japan) according to the manufacturers' instructions. RT-PCR was performed as follows : initial denaturation (95℃) for 2 min, 95℃ for 30 sec, 54℃ for 30 sec, 72℃ for 30 sec, for 30 cycles, followed by a final 5 min extension at 72℃. Primers used in this study were shown in Table 1[4]. Concerning MRP4, primer pair 1 covers all four isoforms of MRP4 and primer pair 2 is specific for isoform 1 (longest one).

Transport activity of [^{14}C] urate

JAR cells were grown in RPMI-1640 medium (Sigma-Aldrich Co., St. Louis, MO, USA) supplemented with 2.0 mg/L $NaHCO_3$ and 10% FBS and JEG-3 cells were grown in Minimum Essential Medium Eagle (Sigma-Aldrich Co., St. Louis, MO, USA) supplemented with 2.2 mg/L $NaHCO_3$ and 10% FBS. The cells were seeded on poly D-lysine coated 24-well plates (1×10^5 cells/well) in the fresh medium and incubated for 48 hours at 37℃ in 5% CO_2. The medium was removed and the cells were washed twice with Hank's balanced salt solution (HBSS) containing 125 mM NaCl, 4.8 mM KCl, 25 mM HEPES, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.3 mM $CaCl_2$ and 5.6 mM glucose (pH 7.4) and further incubated in the same solution at 37℃ for 10 minutes. The uptake of 5 μM [^{14}C] urate in the cells was measured during 30 minutes incubation at 37℃ or 4℃. Uptake was terminated by washing the cells three times with ice-cold HBSS[7]. Then, cells were solubilized with 0.1N NaOH and radioactivity was counted by liquid scintillation spectrometry. The values are expressed as pmol/mg protein/min. For the measurement of the uptake of [^{14}C] urate, four to six wells of the cells were used for each data point. To confirm the reproducibility of the results, three or four separate experiments were performed for each measurement. Results from the representative experiments are shown in the figures.

For urate efflux experiments, JAR and JEG-3 cells were pre-loaded with 5 μM [^{14}C] urate for 30 minutes at 37℃, and then the cells were washed and incubated with or without fumitremorgin C (1 and 5 μM) in the bath solution for 30 minutes. The radioactive both in bath solution and cells were measured to determine the efflux of [^{14}C] urate. Cells were lysed with 0.1 N NaOH and radioactivity was measured using a liquid scintillation counter LSC-7200 (Hitachi Aloka, Inc., Tokyo, Japan). Protein contents of cell lysate were measured by Pierce BCA protein assay kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Data analysis

All experiments were performed in triplicate. Results are presented as mean ± S.E. (n = 4 to 6). Statistical significance was analyzed by using Student's *t*-test for two groups. $p<0.05$ was considered statistically significant.

Table 1 Primers used in conventional RT-PCR

		Transporters Primer sequence (5'-)	Product size (bp)
URAT1	Forward	AACCTCGTGTGTGACTCT	414
	Reverse	AAAGCAGAGGAAGAAGGG	
NPT1	Forward	CCAGATATCCAGGGAATC	416
	Reverse	AGAAGACATACGGCACAG	
NPT4	Forward	GCCCCAAAGAGTCTTCCTGC	505
	Reverse	TCCATGGATAGGAAACGG	
OAT1	Forward	CCGGAAGGTACTCATCTT	334
	Reverse	GGCCGACTCAATGAAGAA	
OAT3	Forward	GGAGGAGCTCAAACTCAA	419
	Reverse	TTGTGTAGAGGAAGAGGC	
OAT4	Forward	GTTCTCGAAGCTCTTGGA	445
	Reverse	CATGAAGATGGACTGGCT	
OAT10	Forward	CCCATCCCTGAAGAATGA	470
	Reverse	AACGTGCAGATTCTGGCA	
URATv1	Forward	GGCCTCAATGCAATTTGG	373
	Reverse	CTGCAATGATGAAGGCAG	
BCRP	Forward	GCAGGATAAGCCACTCAT	432
	Reverse	GACACTCTGTAGTATCCG	
MRP4	Forward 1	TACCAGGAGGTGAAGCCCAA	555
	Reverse 1	TGTCTTCCCCATGGCCATGT	
	Forward 2	TGGTGTGTTCGACAAAGTGC	469
	Reverse 2	GTAAGGCATTCCACAGTTCC	
GAPDH	Forward	GCTGCTTTTAACTCTGGTAA	541
	Reverse	CGCGGCCATCACGCCACAGT	

RESULTS

Expression of urate transporters in JAR and JEG-3 cells

RT-PCR was performed. As shown in Fig. 1, mRNAs for OAT4, BCRP, MRP4 were expressed in JAR cells and mRNAs for OAT10 and BCRP were expressed in JEG-3 cells. Interestingly, breast cancer resistance protein (BCRP/*ABCG2*)[8] mRNA was detected strongly in both cells.

Urate uptake by JAR and JEG-3 cells

[^{14}C] urate uptake by JAR and JEG-3 cells were investigated both at 4℃ and 37℃. Urate uptake at 37℃ were increased in a time-dependent manner, while urate uptake at 4℃ were hardly increased (Fig. 2A). We suspected that transporters were involved in urate uptake of JAR and JEG-3 cells rather than diffusion. To determine whether URAT1[9] and URATv1[10] contribute to this urate uptake, we examined uptake of urate in the presence of benzbromarone, which inhibits URAT1 strongly and URATv1 weakly. JAR and JEG-3 cells were incubated with [^{14}C] urate and 0, 1, 10 and 100 μM benzbromarone for 2 min at 37℃ or 4℃. Urate uptake didn't change throughout the condition. In JEG-3 cells, urate uptake

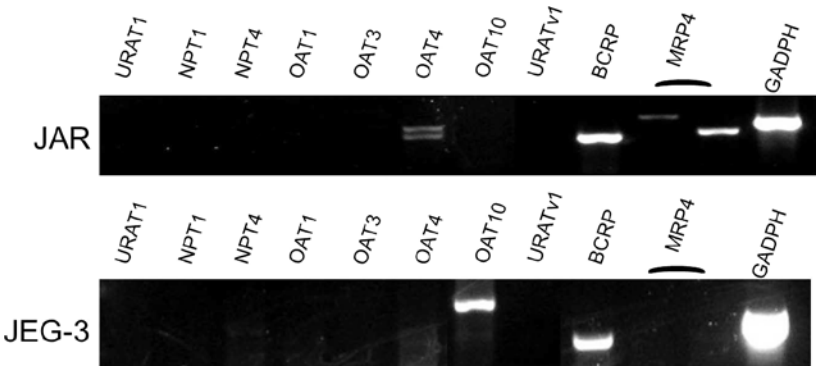

Figure 1 Expression of uric acid transporters in JAR and JEG-3 cells
RT-PCR of urate transporters was performed using collected RNA from JAR and JEG-3 cells. RT-PCR was performed as follows : initial denaturation (95℃) for 2 min, 95℃ for 30 sec, 54℃ for 30 sec, 72℃ for 30 sec, for 30 cycles, followed by a final 5 min extension at 72℃. Expression of each transporter was investigated by the primer pairs listed in Table 1.

with 100 μM benzbromarone was weakly inhibited at 37℃ , indicating that neither URAT1 nor URATv1 contributes to this uptake (Fig. 2B). To identify the responsible transporters in JAR and JEG-3 cells, experiments with Na^+, K^+ and Cl^- substitution were performed. Urate uptake changed only a little in any conditions (Fig. 2C). It suggested that JAR and JEG-3 cells do not have urate transport systems similar to URAT1 nor URATv1.

Urate efflux of JAR and JEG-3 cells

Next, we analyzed the efflux transport of urate in JAR and JEG-3 cells. Urate efflux was observed in both JAR and JEG-3 cells (Fig. 3). To determine whether an ATP-driven efflux transporter BCRP[8] is involed in this step, urate efflux was investigated with or without BCRP inhibitor, fumitremorgin C (FMC)[11]. The efflux of [^{14}C] urate was inhibited by FMC remarkably in both JAR and JEG-3 cells (Fig. 4). Particularly, the efflux of JEG-3 cells was decreased in a dose-dependent manner. These results indicate that [^{14}C] urate efflux via BCRP exists in both JAR and JEG-3 cells.

DISCUSSION

In the present study, we investigated the expression of urate transporters and the properties of urate transport in JAR and JEG-3, human trophoblast-derived cancer cells. Since we found that both JAR and JEG-3 cells express BCRP mRNA strongly and urate efflux was sensitive to BCRP inhibitor FMC, BCRP is suggested to contribute for the permeation of urate produced in placental cells so called syncytiotrophoblasts.

Since urate is water-soluble molecule, it requires a membrane transport protein such as transporter for its permeation of plasma membrane. The identification of URAT1 by Enomoto *et al.* in 2002[9] contributed to advances in the accumulation of information concerning molecules related to renal urate handling together with the identification of new molecules such as OAT4, URATv1, OAT10, OATv1, MRP4 and BCRP involved in urate transport[12,13]. In this study, we demonstrated by conventional PCR that JAR cell expresses mRNAs for BCRP as well as OAT4 and MRP4, and that JEG-3 cell expresses BCRP mRNA together with OAT10. The difference of urate transport properties between JAR and JEG-3 observed in Figs. 2 and 3 may be due to the different expression profile of urate transporters.

As shown in Fig. 2, we found that transporter-mediated uptake of urate in both JAR and JEG-3 cells was based on the comparison of values at 4℃ and 37℃. Our results are different from the one reported previously by Uehara *et al* : they reported that urate moves through the paracellular route by a simple diffusion based on the data from one of the human choriocarcinoma cell lines BeWo[4]. Since JAR and JEG-3

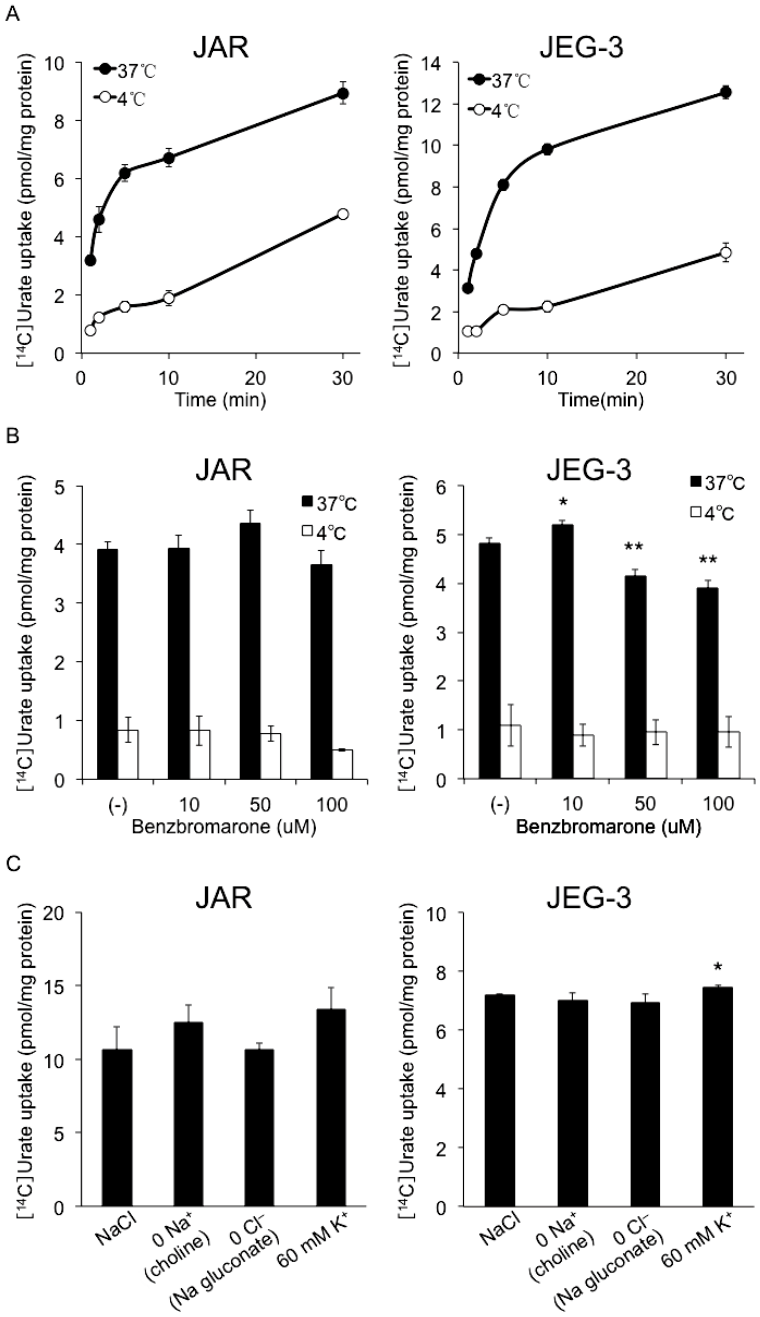

Figure 2 Urate uptake by JAR and JEG-3 cells

A : Time courses of urate uptake were examined at 37℃ (●) and 4℃ (○). Urate uptake of 5 μM [^{14}C] urate in HBSS-added JAR and JEG-3 cells was measured during a 30 min incubation. **B** : Urate uptake was performed in the presence of benzbromarone. JAR and JEG-3 cells were incubated with [^{14}C] urate and 0, 1, 10 and 100 μM benzbromarone for 2 min at 37℃ (■) or 4℃ (□). **C** : Substitution experiments without Na^+, K^+ or Cl^- were analyzed. JAR and JEG-3 cells were incubated with [^{14}C] urate for 5 min in each HBSS. Data are presented as means ± SE for each group. * $p<0.05$, ** $p<0.01$.

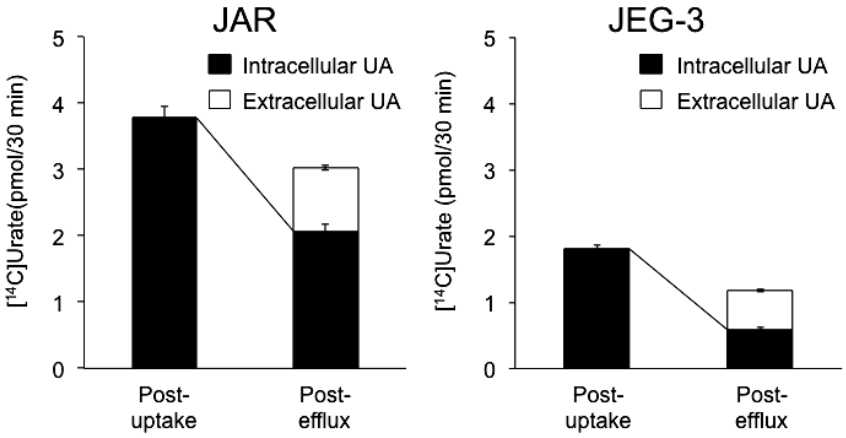

Figure 3 Urate efflux properties in JAR and JEG-3 cells
Efflux of urate from JAR and JEG-3 cells was measured. JAR and JEG-3 cells were pre-incubated with 5 μM [^{14}C] urate for 30 min, and were washed with HBSS. These cells were incubated with new urate-free HBSS for 30 min. After incubation, [^{14}C] urate in the cells (■ : Intracellular UA) and HBSS (□ : Extracellular UA) were measured. Date ara presented as means ± SE.

are used for the model of placental transport of several substances together with BeWo, it seems necessary to evaluate all three cells to conclude the transport characteristics for the model of placental transport.

Urate transport properties of JAR and JEG-3 shown in Fig. 2 did not match to the classical urate transporter characteristics : they lack the dependency to Na^+ and Cl^- and no voltage sensitivity together with no benzbromarone inhibition. These characteristics are completely different from URAT1[9], URATv1[10], OAT4[14], OAT10[15] and OATv1 (formerly NPT4)[16]. Thus we did not expand our research for SLC (solute carrier) transporter and focused on to BCRP[8].

In this study, we found that urate was exported from the cell to the supernatant in both JAR and JEG-3 cells (Fig. 3) and FMC, a BCRP inhibitor, inhibited urate efflux (Fig. 4). These results indicated that efflux of intracellular urate seems to be related to BCRP. Previous report described that blood urate level as well as urate production enzyme xanthine oxidase (XO) level are increased in patients with preeclampsia because of exposed oxidative stress[3]. Therefore, it is suggested that urate is produced in placental cells and accumulated urate is exported by ABC (ATP-binding cassttte) transporter BCRP in the case of preeclampsia. Although the mechanism why hyperuricemia occurs in preeclampsia patients is not clear but, to the best of our knowledge, this is the first report that urate transporter such as BCRP participates urate handling in placenta.

CONCLUSION

In the present study, we investigated mRNA expression of several urate transporters and characterized urate transport in JAR and JEG-3 derived from human trophoblast-derived cells and showed that BCRP, an ATP-driven efflux transporter, contributed the urate handling in placenta, probably for the permeation of urate produced in placental cells to the blood circulation.

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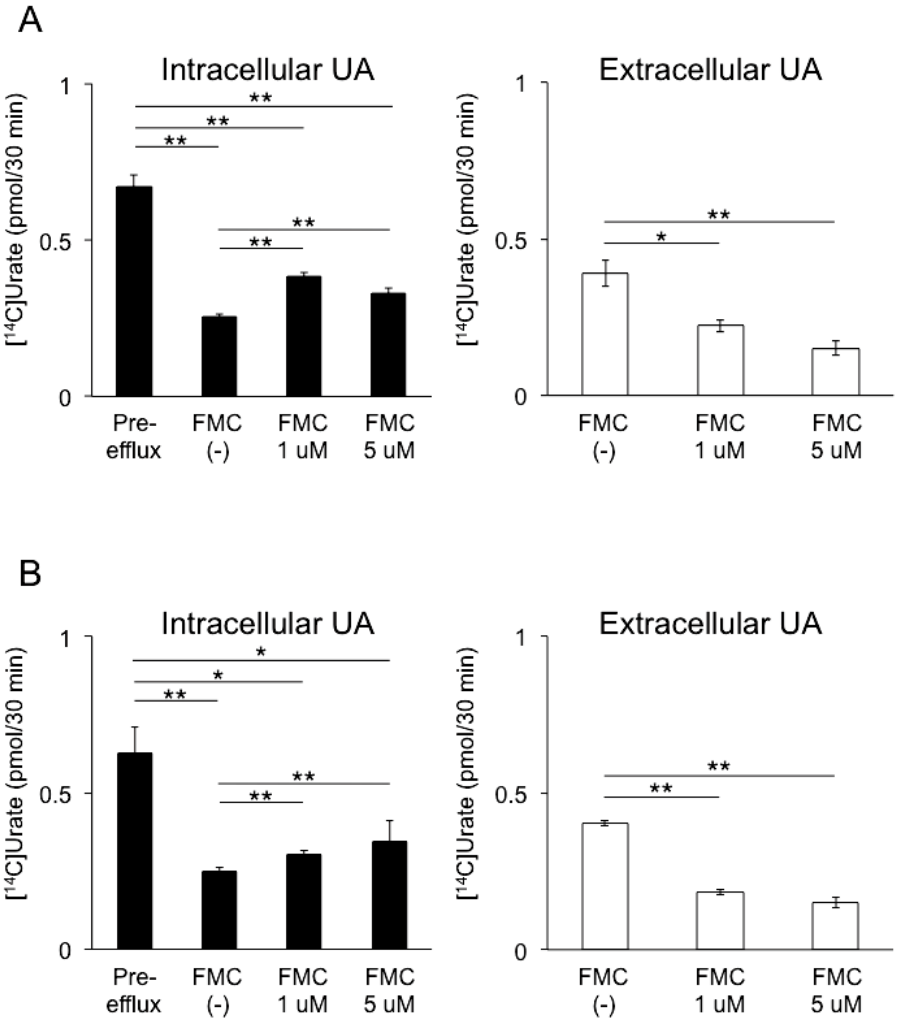

Figure 4 Effect of fumitremorgin C (FMC) on urate efflux
Urate efflux tests were performed in the presence of FMC. After pre-incubation, JAR (**A**) and JEG-3 (**B**) cells were incubated with HBSS for 30 min in the presence of 0, 1 and 5 μM FMC. After incubation, [^{14}C] urate in the cells (Intracellular UA) and HBSS (Extracellular UA) were measured. Data are presented as means ± SE for each group. $^{*}p<0.05$, $^{**}p<0.01$.

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Conflict of Interest.
No COI for all authors.

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