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Effect of hypoxia on UDP-glucuronosyl transferase mRNA expression in human hepatocarcinoma functional liver cell-4 cell line

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Although hypoxic conditions have been reported to affect the expression levels of various enzymes like cytochrome P450, the effect of hypoxia for UDP-glucuronosyl transferase (UGT) expression has been unclear. We evaluated the mRNA expression of UGTs (UGT1A1·1A6·1A9·2B7) in a functional liver cell-4 (FLC-4) cell line by three-dimensional culture under hypoxic conditions (37 °C, 1 % O_2, 5 % CO_2) for 7 days. The mRNA expression of UGT1A1·1A6·1A9·2B7 decreased significantly after 3 days and that of UGT1A1·1A6·1A9 decreased significantly after 7 days. Hypoxic conditions affect the expression levels of UGT enzymes, thus the adjustment of dosage and interval should be considered in drug therapy that metabolized by UGT.

1. Introduction

Hypoxia contributes to the pathophysiology of myocardial and cerebral ischemia, cancer, pulmonary hypertension and chronic obstructive pulmonary disease. Hypoxia can affect the expression levels of drug metabolizing enzymes like cytochrome P450 (CYP) (Fradette and Du Souich 2004). However, there is no report about the expression levels of uridine diphosphate-glucuronosyl transferase (UGT) under hypoxic conditions and the effect of hypoxia on UGTs. The aim of this study was to examine the effect of hypoxia on UGTs mRNA expression levels.

2. Investigations and results

After 1 day under hypoxic conditions, UGT1A1.1A6 mRNA expression was significantly increased, compared to the expression under normoxia (Fig. 1, $P < 0.05$). After 3 days under hypoxic conditions, UGT1A1.1A6.1A9.2B7 mRNA expression was significantly decreased compared to the expression under normoxia (Fig. 1, $P < 0.05$). After 7 days under hypoxic conditions, UGT1A1.1A6.1A9 mRNA expression was significantly decreased compared to the expression under normoxia (Fig. 1, $P < 0.05$). UGT2B7 mRNA expression was significantly increased after 7 days under hypoxic conditions compared to the expression under normoxia (Fig. 1, $P < 0.05$).

In the present study, human hepatocellular carcinoma FLC-4 cells cultured in a three-dimensional configuration were used. Earlier, we reported that FLC-4 cells cultured in three-dimensional configuration showed significantly higher expression levels of UGTs than HepG2 cells (Kato et al. 2014). Although hypoxia contributes to the pathophysiology of human diseases, the effect of hypoxia for UGT enzymes has not been clear. The present study revealed that the mRNA expression of UGT1A1.1A6.1A9 was significantly decreased and that of UGT2B7 was significantly increased after 7 days under hypoxic conditions, in comparison with the expressions under normoxia. This is the first report about the effect of hypoxia to mRNA expression of the UGTs.

3. Discussion

In our previous study, the mRNA expression of HNF-4α was associated with those of UGT1A1.1A6.1A9.2B7 (Kato et al. 2014). In the present study, the mRNA expression of HNF-4α was significantly decreased after 3 days and increased after 7 days (Fig. 2). The behavior of the mRNA expression levels of UGT2B7 was different from that of HNF-4α after 7 days. This phenomenon indicated that some factor except HNF-4α might regulate the mRNA expression of UGT2B7 under hypoxic conditions. In this study, we only measured the mRNA expression levels of drug-metabolizing enzymes and did not evaluate their activity. It has been previously reported that good correlations exist between mRNA expression levels and enzyme activities of UGTs (Richert et al. 2009). Therefore, the results of this study should roughly reflect the enzyme activities of UGTs. This study revealed that hypoxic conditions affect the expression levels of UGT enzymes, thus the adjustment of dosage and interval should be considered in drug therapy that metabolized by UGT.

4. Experimental

4.1. Three-dimensional culture of FLC-4

FLC-4 cells [previous names JHH-4 (JCRB0435) (Hasumura et al. 1988)] obtained from the Health Science Research Resources Bank (Osaka, Japan) were grown and maintained in Dulbecco's Modified Eagle Medium

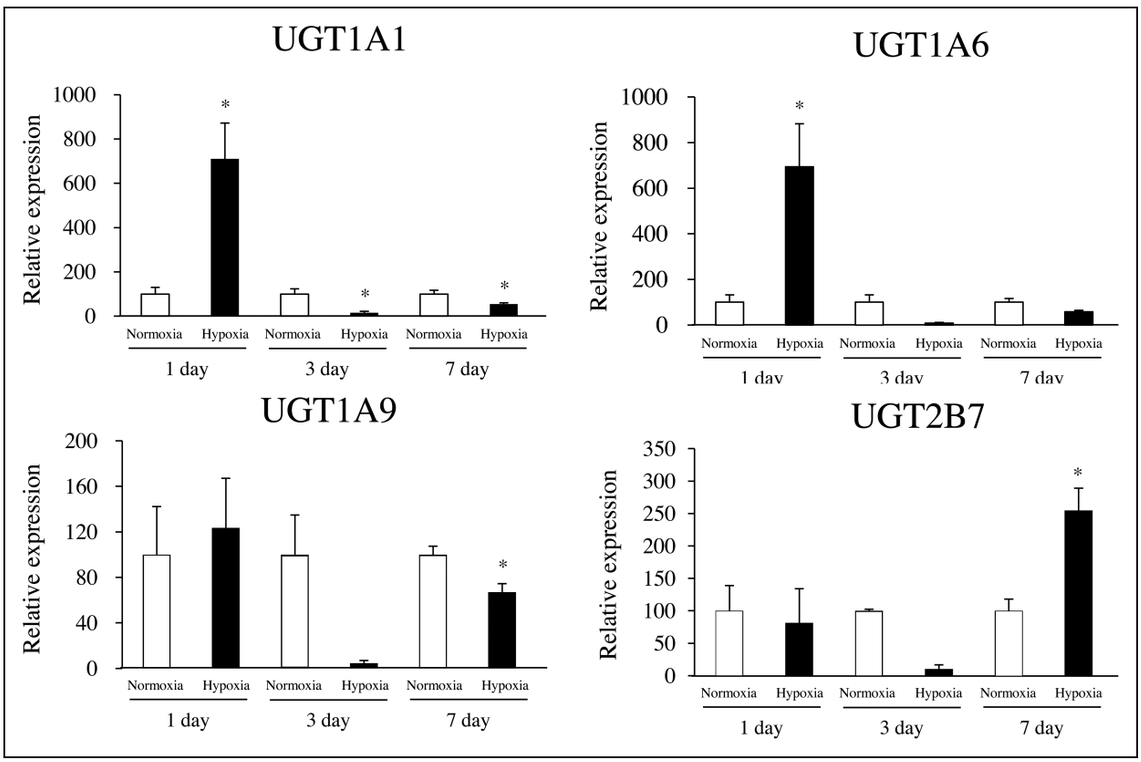

Fig. 1: UGT1A1, UGT1A6, UGT1A9, and UGT2B7 mRNA expression in FLC-4 cells. mRNA expression levels were determined using real-time PCR. FLC-4 cells in three-dimensional culture were seeded at a density of 1.5×10^5 cells/well on conventional cell culture plates and cultured for 1, 3, and 7 days. The mRNA expression levels of UGTs were normalized to that of 18S ribosomal RNA (18S rRNA). 18S, 18S rRNA. Data are shown as mean ± SE (n = 3–4). $^{*}P < 0.05$, $^{**}P < 0.01$, compared to normoxia.

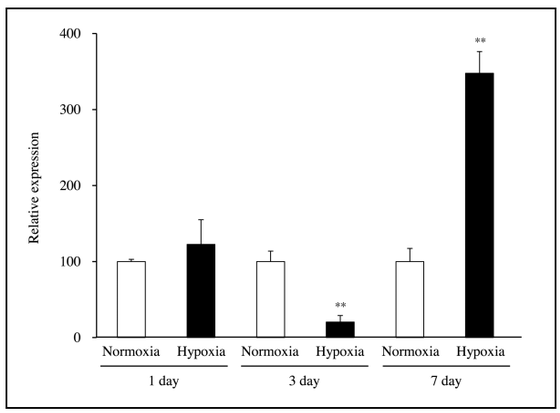

Fig. 2: HNF-4α mRNA expression in FLC-4 cells. mRNA expression levels were determined using real-time PCR. FLC-4 cells in three-dimensional culture were seeded at a density of 1.5×10^5 cells/well on conventional cell culture plates and cultured for 1, 3, and 7 days. The expression levels of *HNF4A* mRNA were normalized to that of 18S ribosomal RNA (18S rRNA). 18S, 18S rRNA. Data are shown as mean ± SE (n = 3–4). $^{*}P < 0.05$, $^{**}P < 0.01$, compared to normoxia.

(DMEM, 4.5 g glucose/L, Invitrogen Co., CA, USA), containing 10% fetal bovine serum (FBS, BioSource International Inc., CA, USA), penicillin (50 IU/mL) and streptomycin (50 IU/mL) (PS, Life Technologies Corporation, Carlsbad, CA, USA) at 37 °C with 5 % CO_2 in 25 cm^2 tissue culture flasks (Becton-Dickinson Ind., NJ, USA). The FLC-4 cell line were subcultured by treatment with 0.25% trypsin-1 mM ethylene diamine tetra acetic acid (EDTA) in Ca^{2+} and Mg^{2+} free phosphate-buffered saline (PBS) solution every 3 days. The cells were then detached from the stock cultures by trypsin digestion, washed once by centrifugation. FLC-4 (5×10^6 cells/mL) and growth factor-reduced Matrigel (Becton Dickinson, Lincoln Park, NJ) were mixed in equal amount and placed on a 24-well multiplate for 30 min at 37 °C with 5 % CO_2. One mL of DMEM with 10 % FBS and 1 % PS was added each well and cultured under 37 °C, 5 % CO_2, 20 %O_2 (normoxia) and 37 °C, 5 % CO_2, 1 % O_2 (hypoxia) for 7 days.

4.2. RNA isolation and analysis

Total RNA was extracted from FLC-4 using TRI reagent® (Molecular Research Center, Inc., Cincinnati, OH, United States) on days 1, 3, and 7. cDNA was synthesized by reversing transcription using total RNA solution (Transcriptor Universal cDNA Master, Roche Diagnostics, Basel, Switzerland). The primers and probe were designed according to the nucleotide sequence of the human UGT1A1.1A6.1A9.2B7, HNF-4α, and 18 s ribosomal RNA (rRNA) using Probefinder v2.45 (http://qpcr.probefinder.com/roche3.html). This online software specifies a set of specific primers plus the TaqMan locked nucleic acid probe from the Roche Universal Probe Library collection. Universal Probe #22 for UGT2B7, #77 for HNF-4α and 18 s rRNA, and #78 for UGT1A1.1A6.1A9 were purchased from Roche Diagnostics. mRNA measurement was performed using the LightCycler® (Roche Diagnostics, Basle, Switzerland). The results were adjusted using 18 s rRNA, a housekeeping gene, as the internal standard substance.

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