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Research Paper

Thyroid Hormone Regulates the Expression and Function of P-glycoprotein in Caco-2 Cells

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Purpose. In patients with thyroid disorders, abnormalities in the pharmacokinetics of various drugs including digoxin, a substrate of P-glycoprotein (Pgp) which plays a crucial role in drug absorption and disposition, have been reported. In this study, we examined the effect of 3,5,3′-L-triiodothyronine (T_3) on the function and expression of Pgp using the human intestinal epithelial cell line Caco-2.
Materials and Methods. The effect of T_3 on the expression of Pgp and MDR1 mRNA was assessed by Western blotting and competitive polymerase chain reaction, respectively. Digoxin uptake and transport by Pgp was assessed using Caco-2 cell monolayers.
Results. The expression of MDR1 mRNA was increased by T_3 treatment in a concentration-dependent manner. Pgp expression was also increased by 100 nM T_3, whereas it decreased on depletion of T_3. The amount of [^{3}H]digoxin accumulated in Caco-2 cell monolayers treated with T_3 was diminished significantly compared with that in control cells. In addition, the basal-to-apical transcellular transport of [^{3}H]digoxin was accelerated by T_3 treatment.
Conclusions. These results indicate that T_3 regulates the expression and function of Pgp. It is possible that changes in Pgp expression alter the pharmacokinetics of Pgp substrates in patients with thyroid disorders.

KEY WORDS: Caco-2 cells; digoxin; P-glycoprotein; 3,5,3′-L-triiodothyronine.

INTRODUCTION

P-glycoprotein (Pgp), a 170 kDa membrane glycoprotein and gene product of MDR1, acts as an ATP-dependent multidrug efflux pump which transports a wide range of hydrophobic compounds such as β-blockers, calcium channel antagonists, anticancer agents, and immunosupressants. Expression of Pgp in humans and rodents is observed in various tissues including the brain, liver, kidney, and small intestine. Therefore, Pgp is considered to be closely related to the absorption, distribution, and excretion of drugs, suggesting that the alteration of Pgp expression levels may affect the pharmacokinetics of drugs (1). Greiner *et al.* (2) showed that the AUC of digoxin, a Pgp substrate, was decreased following an up-regulation of Pgp expression caused by the coadministration of rifampin. Dexamethasone also modulates Pgp expression and affects the pharmacokinetics of Rhodamine 123 in rats (3,4). Pharmacokinetic variations due to changes in Pgp expression levels may be closely related to the efficacy and side effects of drugs.

Thyroid hormone is secreted from the thyroid gland to maintain normal growth, development, body temperature, and energy levels. Most of its effects appear to be mediated by the activation of nuclear receptors that regulate mRNA transcription and subsequent protein synthesis. A change in the serum thyroid hormone level may be followed by the altered expression of proteins that have important physiological functions. In a hyperthyroid state, in addition to an increase in appetite and a reduction in body weight, the pharmacokinetics of drugs such as propranolol (5) and digoxin (6–9) changed dramatically. We previously demonstrated that thyroid hormone induced Pgp and mdr1a/1b mRNA expression in hyperthyroid rats (10). Furthermore, Siegmund *et al.* (11) showed that the administration of levothyroxine tended to up-regulate the expression of MDR1 mRNA and Pgp in healthy volunteers. However, it did not result in major alterations to the pharmacokinetics of talinolol. Therefore, the mechanism of the changes in pharmacokinetics of drugs caused by thyroid hormone remains to be elucidated.

In the present study, to evaluate in further detail the effect of thyroid hormone in the small intestine, we investigated the effect of thyroid hormone on the expression of MDR1 mRNA and Pgp abundance and Pgp function using the human intestinal epithelial cell line Caco-2.

MATERIALS AND METHODS

Materials. [^{3}H]Digoxin (1.37 TBq/mmol) was obtained from PerkinElmer Life and Analytical Sciences (Boston, MA). [^{14}C]Inulin (259 MBq/mmol) was from Moravek Biochemicals Inc.

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ABBREVIATIONS: PCR, polymerase chain reaction; Pgp, P-glycoprotein; T_3, 3,5,3′-L-triiodothyronine.

(Brea, CA). 3,5,3′-L-triiodothyronine (T_3) was purchased from Nacalai Tesque (Kyoto, Japan). AG-1-X8 anion exchange resin (chloride form; 200-400 mesh) was obtained from Bio-Rad (Hercules, CA). All other chemicals were of the highest purity available.

Cell Culture. Caco-2 cells at *Passage 18* obtained from the American Type Culture Collection (ATCC HTB-37) were maintained by serial passage in plastic culture dishes as described previously (12). For uptake experiments, 35-mm plastic culture dishes were inoculated with 2×10^5 cells in 2 ml of complete culture medium. The medium consisted of DMEM (Sigma) supplemented with 10% fetal bovine serum (Whittaker Bioproducts, Walkersville, MD) and 1% non-essential amino acids (Invitrogen Life Technologies, Carlsbad, CA) without antibiotics before T_3 treatment. Cells were used for experiments on the 15th day after seeding. In this study, Caco-2 cells were used between *Passages 35* and *48*.

Cell Treatment. A stock solution of T_3 was prepared as a 1 mM solution in 0.1 M NaOH. For T_3 treatment, serum was treated with anion exchange resin AG-1-X8 to remove the thyroid hormone according to the method of Samuels *et al.* (13). The T_3 concentration in the treated serum was below the level of detection (<0.15 ng/ml) of an enzyme immunoassay method (Imx; Dainabot, Tokyo, Japan). To expose the Caco-2 cell monolayers to T_3, we used a culture medium containing T_3-depleted serum and 100 nM T_3. T_3 treatment was applied to post-confluent monolayers. The control cells were incubated with the same concentration of 0.1 M NaOH in each experiment.

Measurements of Cellular Accumulation and Transcellular Transport. The cellular accumulation and transcellular transport of [^{3}H]digoxin were measured using monolayer cultures grown on 35-mm culture dishes and Transwell™ cell chambers (Costar, Cambridge, MA), respectively. The composition of the incubation medium was as follows: 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose, and 5 mM HEPES (pH 7.4).

The accumulation of [^{3}H]digoxin was studied according to the method of Ashida *et al.* (12). Radioactivity was measured in 3 ml of ACS II (Amersham Pharmacia Biotech) by liquid scintillation counting. The protein contents of cell monolayers solubilized in 1 N NaOH were determined using a Bio-Rad protein assay kit with bovine γ -globulin as the standard.

For transcellular transport experiments, after removal of the culture medium from both sides of the monolayers, the cell monolayers were preincubated with 2 ml of incubation medium at 37°C for 10 min. Then, 2 ml of incubation medium containing [^{3}H]digoxin and [^{14}C]inulin was added to either the basolateral or the apical side, with 2 ml of non-radioactive incubation medium added to the opposite side for specified periods at 37°C. After the incubation, aliquots (50 μl) of the incubation medium on the other side were taken at specified time points, and the radioactivity of [^{3}H]digoxin and [^{14}C]inulin was measured. [^{14}C]inulin was used for the correction of paracellular transport.

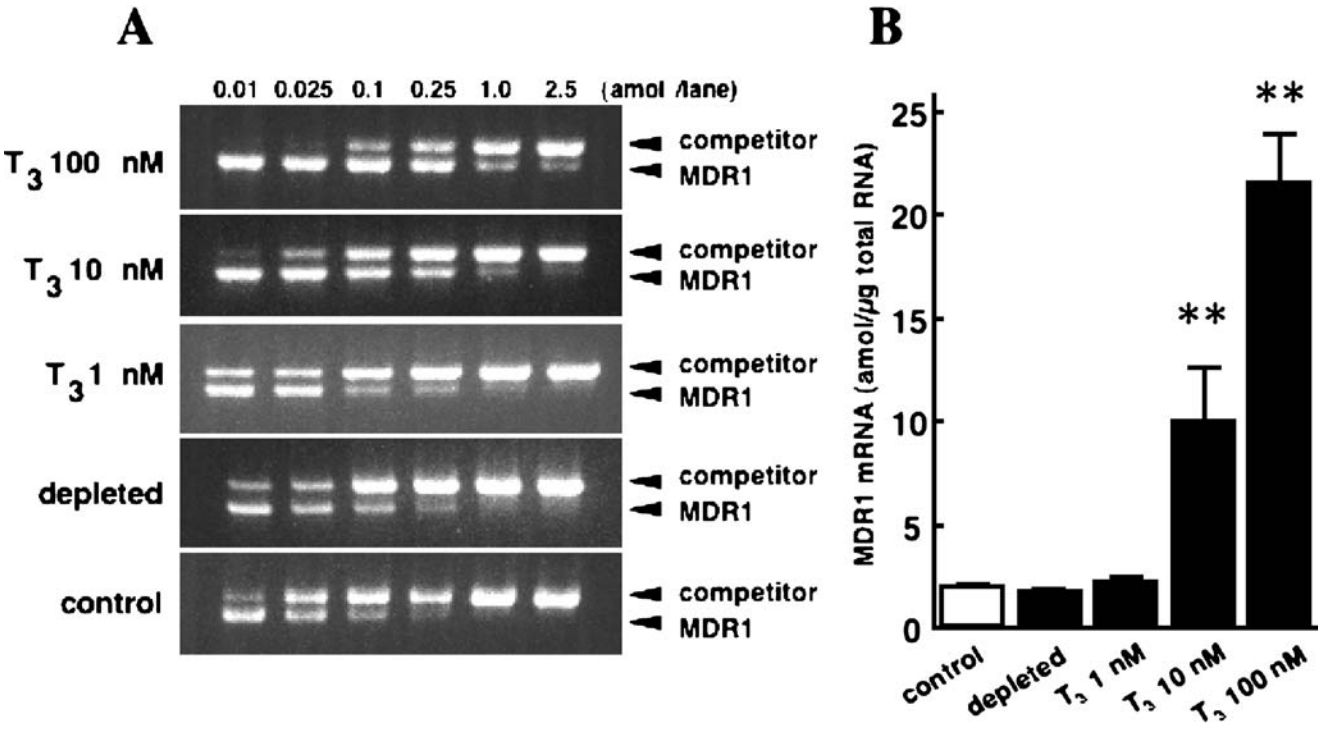

Fig. 1. Dose-dependent effects of T_3 on the expression of MDR1 mRNA in Caco-2 cells. Cells were treated with various concentrations of T_3 (depleted, 1 nM, 10 nM, 100 nM) for 3 days. Depleted represents treatment with the culture medium removed the thyroid hormone from serum by anion exchange resin AG-1-X8. After treatment, total RNA was isolated and competitive PCR was performed to determine MDR1 mRNA levels. **A** Typical results of agarose gel electrophoresis of the PCR products from T_3-treated cells. **B** Densitometric quantification of MDR1 mRNA, corrected using the amount of GAPDH mRNA as an internal control. Each column represents the mean±SE of six monolayers. *Double asterisk*, $P<0.01$, significantly different from the control.

Competitive Polymerase Chain Reaction (PCR). Competitive PCR was performed according to the method of Masuda *et al.* (14) with some modifications. Aliquots of 1 μg of total cellular RNA, isolated from Caco-2 cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany), were reverse-transcribed in 20 μl of diluted reaction mixture and diluted to 200 μl. Aliquots of 5 μl of diluted reaction mixture, in combination with a semi-logarithmic serial dilution of mimic competitor DNA from 100 to 0.01 amol, were amplified by PCR according to the following method: 5 μM human MDR1 sense primer and 5 μM antisense primer in 20 μl were incubated according to the following PCR profile: an initial denaturation step of 95°C for 3 min followed by the cycling program, 95°C for 1 min, 65°C for 1 min, and 72°C for 1 min, and a final elongation step of 72°C for 10 min. For each primer set, the number of PCR cycles was increased under otherwise fixed conditions to determine the point halfway through the exponential phase. The number of cycles was 34 for MDR1. PCR products were then sized-fractionated by 1.5% agarose gel electrophoresis. The amplified cellular fragments of MDR1 were 546 bp, and the mimic competitor was 604 bp. The amount of competitor DNA yielding equal molar amounts of product gave that of the target MDR1 mRNA.

Western Blot Analysis. The apical membrane fraction from Caco-2 cells was isolated as described previously (12). After blotting onto Immobilon-P membranes (Millipore, Bedford, MA), the monoclonal antibody C219 (CIS Bio International, Gif-sur-Yvette, France) and a polyclonal antibody to villin (Santa Cruz Biotechnology, Santa Cruz, CA) were used to detect the expression of P-gp and villin, respectively. The relative densities of the bands in each lane were determined using NIH Image 1.61 (National Institutes of Health, Bethesda, MD), and the densitometric ratio of Pgp to that of villin was calculated.

Statistical Analysis. Data were analyzed statistically with a non-paired *t* test. Probability values of less than 5% were considered significant. In the mRNA analysis by Competitive PCR, statistical analysis was performed with the one-way ANOVA followed by Dunnett's post hoc testing.

RESULTS

mRNA Analysis by Competitive PCR. We used the serum treated with anion exchange resin to deplete thyroid hormone and then added various concentrations of T_3. Figure 1 shows the effect of various concentrations of T_3 (1 nM to 100 nM or depletion) on the expression of MDR1 mRNA in Caco-2 cells. The expression levels of MDR1 mRNA were increased by T_3 pretreatment for 3 days in a concentration-dependent manner.

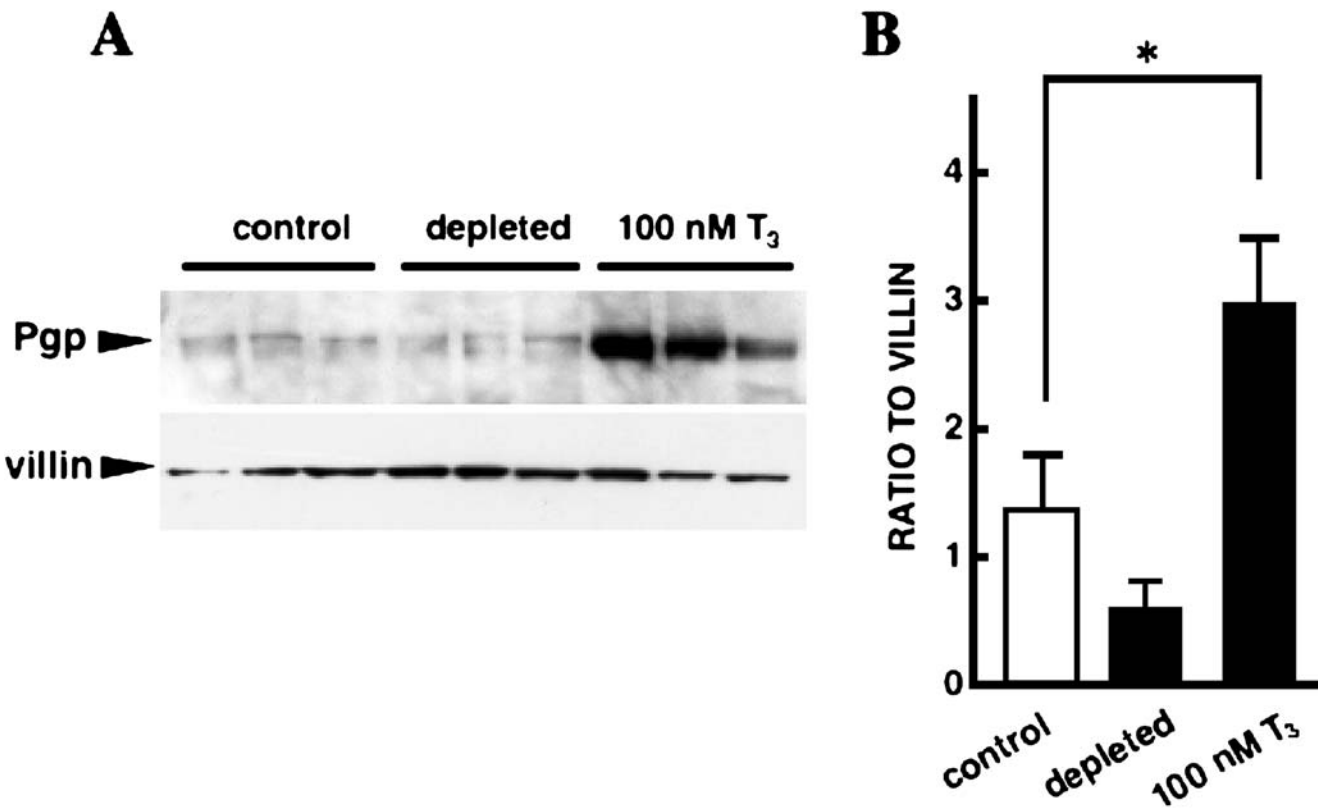

Fig. 2. Western blot analysis of the apical membranes from Caco-2 cells for Pgp. Apical membranes (50 μg) from Caco-2 cells were separated by SDS-PAGE (7.5%) and blotted onto a polyvinylidene difluoride membrane. The monoclonal antibody C219 (200 ng/ml) and a polyclonal antibody to villin (1:1,000) were used to detect the expression of Pgp and villin as primary antibodies. A horse radish peroxidase-conjugated anti-mouse IgG antibody and anti-goat IgG antibody were used for detection of bound antibodies, and strips of blots were visualized by chemiluminescence on X-ray film. **A** Immunoblotting of apical membranes from Caco-2 cells treated with 100 nM T_3 or without T_3 (depleted). **B** Densitometric quantification of Pgp. The level of C219 was corrected using villin as an internal standard. Each column represents the mean ± SE of three samples. *Asterisk*, $P<0.05$, significantly different from the control.

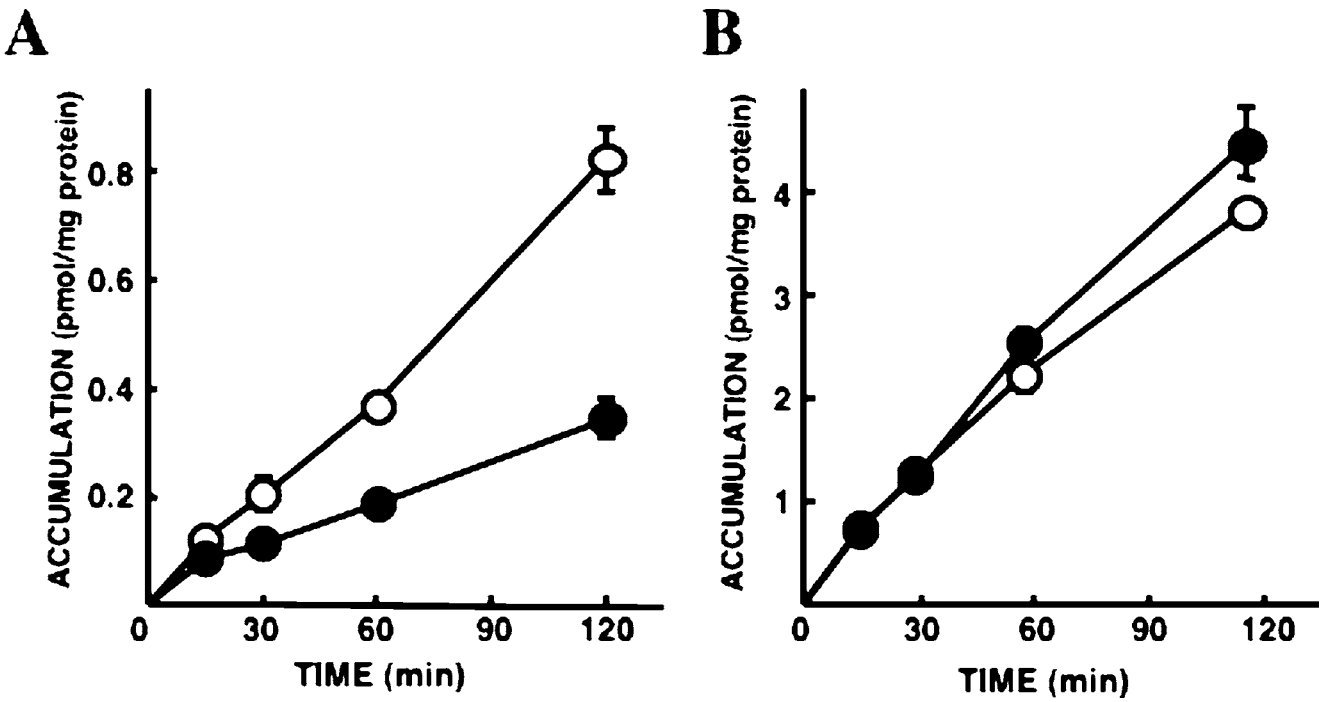

Fig. 3. Effects of T_3 on the uptake of [^{3}H]digoxin by Caco-2 cells in the **A** absence or **B** presence of 10 μM cyclosporin A. The uptake of [^{3}H]digoxin by Caco-2 cells treated with 100 nM T_3 (closed circle) or without T_3 (open circle) for 3 days was measured for specified periods at 37°C. Each point represents the mean±SE of nine monolayers from three separate experiments.

Western Blotting. Then Western blotting was performed to investigate the effect of T_3 on the expression of Pgp in Caco-2 cells (Fig. 2). A significant increase in Pgp expression was observed in Caco-2 cells pretreated with 100 nM T_3 for 3 days. In contrast, the expression level was decreased by half on the depletion of T_3.

Effect of T_3 Pretreatment on Cellular Accumulation and Transcellular Transport of [^{3}H]digoxin in Caco-2 Cells. To investigate whether the transport activity of Pgp was altered by T_3 pretreatment in Caco-2 cells, we performed [^{3}H]digoxin uptake experiments. As shown in Fig. 3, the uptake of digoxin was decreased significantly by T_3 pretreatment. In the presence of 10 μM cyclosporin A, an inhibitor of Pgp, the amount of digoxin accumulated in T_3-treated cells did not differ from that in non-pretreated cells. Figure 4 shows the transcellular transport of digoxin across Caco-2 cell monolayers. The transcellular transport of digoxin from the apical to basolateral side of Caco-2 cell monolayers treated with 100 nM T_3 was very low similar to the control. On the other hand, the basolateral to apical transcellular transport of digoxin was significantly increased across Caco-2 cell monolayers treated with 100 nM T_3. These results indicated that T_3 pretreatment caused the stimulation of Pgp-mediated transport following a significant increase in Pgp expression.

DISCUSSION

Earlier investigations suggested that the expression of Pgp varies in response to several factors. Westphal *et al.* (15) showed that treatment with rifampin resulted in an increase in the expression of duodenal Pgp and MDR1 mRNA, and Pgp expression significantly correlated with the systemic clearance of intravenous talinolol. In addition, it was reported that St John's Wort induced intestinal Pgp expression in rats

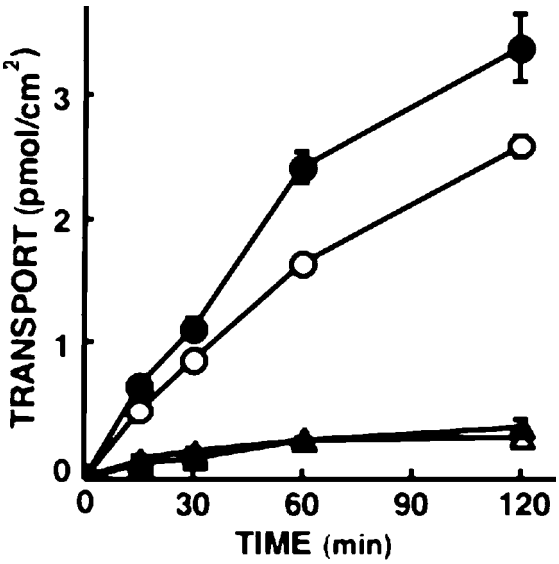

Fig. 4. Effects of T_3 on the transcellular transport of [^{3}H]digoxin using Caco-2 cells. The transcellular transport of [^{3}H]digoxin by Caco-2 cells treated with 100 nM T_3 (*closed symbol*) or without T_3 (*open symbol*) for 3 days was measured. [^{3}H]Digoxin was added to either the basolateral (*circle*) or the apical (*triangle*) side of Caco-2 cells, and incubated for specified periods at 37°C. After the incubation, the radioactivity in the medium of the opposite site was measured. Each point represents the mean±SE of nine monolayers from three separate experiments.

and humans (16). Cyclosporin A treatment also induced the expression of Pgp in the kidney and other tissues in rats (17). We previously reported that levels of Pgp and mdr1a/1b mRNA were increased in the hyperthyroid rat kidney, liver, and intestine (10). Western blot analysis revealed that Pgp expression was markedly increased in the kidney and liver of hyperthyroid rats. In contrast, it was slightly increased in the jejunum and ileum. In the present study, however, we showed that treatment with T_3 resulted in significantly increased levels of Pgp and MDR1 mRNA in Caco-2 cells. Several reports have indicated a cell type- or species-specific regulation of mdr gene expression. Zhao *et al.* showed that MDR1 mRNA levels were elevated in dexamethasone-treated $HepG_2$ cells, a human hepatoma cell line, but not in nonhepatoma HeLa cells (18). In addition, Chin *et al.* demonstrated that exposure to several drugs increased mdr RNA levels substantially in rodent cells, but not human cells (19). Thus, it is suggested that species differences exist in the susceptibility to Pgp induction. Further studies are needed to elucidate the precise mechanism of transcriptional regulation of MDR1 mRNA by thyroid hormone.

Thyroid hormones have a diverse range of actions including effects on differentiation and development, thermogenesis, and metabolism. Therefore, pathologic abnormalities in serum thyroid hormone levels result in physiological changes. For example, patients with hyperthyroidism often exhibit weight loss, a low cholesterol level, an elevated body temperature, and tachycardia, whereas hypothyroidism provokes hypercholesterolemia, myxedema, and bradycardia (20). It is known that thyroid hormone activates nuclear receptors as a ligand, leading to mRNA expression and subsequent protein synthesis. Therefore, changes in serum thyroid hormone levels may affect the expression of proteins that are of physiological importance.

Previous studies have shown that thyroid hormone affects the expression level of several membrane transporters such as the peptide transporter PEPT1 (12,21), the glucose transporter GLUT4 (22), the fructose transporter GLUT5 (23), the ATP-binding cassette transporter ABCA1 (24), Na^+, K^+-ATPase (25), and the Na^+/H^+ exchanger NHE1 (26). In addition, Siegmund *et al.* (11) showed that administration of levothyroxine tended to induce the up-regulation of MDR1 mRNA and Pgp expression in healthy volunteers. However, it did not result in major alterations in the pharmacokinetics of talinolol because their group administered levothyroxine in doses that did not cause thyrotoxicosis. In the present study, we showed that in Caco-2 monolayers treated with 100 nM T_3, the accumulation of [^{3}H]digoxin was decreased significantly and the basal-to-apical transcellular transport of [^{3}H]digoxin was accelerated compared with that in control cells. In the hypothyroid state, no change of MDR1 mRNA expression was observed compared to the control. On the other hand, the Pgp expression level decreased by half on depletion of T_3 and the amount of [^{3}H]digoxin accumulated increased slightly (1.27- to 1.50-fold). Therefore, it is possible that the mechanisms behind the regulation of Pgp expression by T_3 differ in the hyperthyroid *versus* hypothyroid.

In the present study, the altered expression of Pgp was correlated with the transport activity of digoxin in Caco-2 cells. The findings correspond to clinical reports that the blood concentration of digoxin is decreased in hyperthyroidism and increased in hypothyroidism. In contrast, there are several reports indicating the lack of pharmacokinetic alterations in the hyperthyroidism (27). For example, Ochs *et al.* (28) reported that the kinetics of diazepam were not altered in patients with hyperthyroidism. However, diazepam is not a substrate for Pgp and the metabolism by CYP2C19 and CYP3A4 are considered to be main pathways in the elimination of diazepam from the body. Therefore, it is likely that the kinetics of diazepam are not affected with hyperthyroidism. As for Pgp substrates, it was recently reported that long-term levothyroxine treatment decreased the oral bioavailability of cyclosporine A (29). Thus, it is reasonable to assume that the changes in Pgp expression in the human gut affect at least in part the alteration of the blood concentration of digoxin. Although it is impossible to investigate the changes in Pgp expression in other human tissues for ethical reasons, urinary excretion of digoxin might be increased if the expression of Pgp could be induced in the human kidney.

During the course of this study, Mitin *et al.* (30) reported that levothyroxine up-regulated the expression of Pgp mRNA and protein *in vitro*. Their observations were consistent with the present finding that treatment with T_3 resulted in the inducible expression of Pgp and MDR1 mRNA in Caco-2 cells. In addition, we further demonstrated that the basal-to-apical transcellular transport of [^{3}H]digoxin was accelerated by T_3 treatment. However, the precise molecular mechanisms underlying the induction of Pgp expression by T_3 remain to be clarified.

In conclusion, we demonstrated that thyroid hormone regulates the expression and function of Pgp. It is possible that changes in Pgp expression alter the pharmacokinetics of Pgp substrates in patients with thyroid disorders.

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REFERENCES

1. J. H. Lin and M. Yamazaki. Role of P-glycoprotein in pharmacokinetics: clinical implications. *Clin. Pharmacokinet.* **42**:59–98 (2003).
2. B. Greiner, M. Eichelbaum, P. Fritz, H. P. Kreichgauer, O. von Richter, J. Zundler, and H. K. Kroemer. The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J. Clin. Invest.* **104**:147–153 (1999).
3. M. Demeule, J. Jodoin, E. Beaulieu, M. Brossard, and R. Beliveau. Dexamethasone modulation of multidrug transporters in normal tissues. *FEBS Lett.* **442**:208–214 (1999).
4. S. Micuda, L. Mundlova, J. Mokry, J. Osterreicher, J. Cermanova, D. Cizkova, and J. Martinkova. The effect of mdr1 induction on the pharmacokinetics of rhodamine 123 in rats. *Basic Clin. Pharmacol. Toxicol.* **96**:257–258 (2005).
5. P. G. Wells, J. Feely, G. R. Wilkinson, and A. J. Wood. Effect of thyrotoxicosis on liver blood flow and propranolol disposition after long-term dosing. *Clin. Pharmacol. Ther.* **33**:603–608 (1983).

6. J. R. Lawrence, D. J. Sumner, W. J. Kalk, W. A. Ratcliffe, B. Whiting, K. Gray, and M. Lindsay. Digoxin kinetics in patients with thyroid dysfunction. *Clin. Pharmacol. Ther.* **22**:7–13 (1977).
7. G. M. Shenfield, J. Thompson, and D. B. Horn. Plasma and urinary digoxin in thyroid dysfunction. *Eur. J. Clin. Pharmacol.* **12**:437–443 (1977).
8. J. Bonelli, H. Haydl, K. Hruby, and G. Kaik. The pharmacokinetics of digoxin in patients with manifest hyperthyroidism and after normalization of thyroid function. *Int. J. Clin. Pharmacol. Biopharm.* **16**:302–306 (1978).
9. G. M. Shenfield. Influence of thyroid dysfunction on drug pharmacokinetics. *Clin. Pharmacokinet.* **6**:275–297 (1981).
10. N. Nishio, T. Katsura, K. Ashida, M. Okuda, and K. Inui. Modulation of P-glycoprotein expression in hyperthyroid rat tissues. *Drug Metab. Dispos.* **33**:1584–1587 (2005).
11. W. Siegmund, S. Altmannsberger, A. Paneitz, U. Hecker, M. Zschiesche, G. Franke, W. Meng, R. Warzok, E. Schroeder, B. Sperker, B. Terhaag, I. Cascorbi, and H. K. Kroemer. Effect of levothyroxine administration on intestinal P-glycoprotein expression: consequences for drug disposition. *Clin. Pharmacol. Ther.* **72**:256–264 (2002).
12. K. Ashida, T. Katsura, H. Motohashi, H. Saito, and K. Inui. Thyroid hormone regulates the activity and expression of the peptide transporter PEPT1 in Caco-2 cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* **282**:G617–623 (2002).
13. H. H. Samuels, F. Stanley, and J. Casanova. Depletion of L-3,5,3′-triiodothyronine and L-thyroxine in euthyroid calf serum for use in cell culture studies of the action of thyroid hormone. *Endocrinology* **105**:80–85 (1979).
14. S. Masuda, S. Uemoto, T. Hashida, Y. Inomata, K. Tanaka, and K. Inui. Effect of intestinal P-glycoprotein on daily tacrolimus trough level in a living-donor small bowel recipient. *Clin. Pharmacol. Ther.* **68**:98–103 (2000).
15. K. Westphal, A. Weinbrenner, M. Zschiesche, G. Franke, M. Knoke, R. Oertel, P. Fritz, O. von Richter, R. Warzok, T. Hachenberg, H. M. Kauffmann, D. Schrenk, B. Terhaag, H. K. Kroemer, and W. Siegmund. Induction of P-glycoprotein by rifampin increases intestinal secretion of talinolol in human beings: a new type of drug/drug interaction. *Clin. Pharmacol. Ther.* **68**:345–355 (2000).
16. D. Durr, B. Stieger, G. A. Kullak-Ublick, K. M. Rentsch, H. C. Steinert, P. J. Meier, and K. Fattinger. St John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. *Clin. Pharmacol. Ther.* **68**:598–604 (2000).
17. L. Jette, E. Beaulieu, J. M. Leclerc, and R. Beliveau. Cyclosporin A treatment induces overexpression of P-glycoprotein in the kidney and other tissues. *Am. J. Physiol.* **270**:F756–765 (1996).
18. J. Y. Zhao, M. Ikeguchi, T. Eckersberg, and M. T. Kuo. Modulation of multidrug resistance gene expression by dexamethasone in cultured hepatoma cells. *Endocrinology* **133**:521–528 (1993).
19. K. V. Chin, S. S. Chauhan, I. Pastan, and M. M. Gottesman. Regulation of mdr RNA levels in response to cytotoxic drugs in rodent cells. *Cell Growth Differ.* **1**:361–365 (1990).
20. R. C. Ribeiro, J. W. Apriletti, B. L. West, R. L. Wagner, R. J. Fletterick, F. Schaufele, and J. D. Baxter. The molecular biology of thyroid hormone action. *Ann. N. Y. Acad. Sci.* **758**:366–389 (1995).
21. K. Ashida, T. Katsura, H. Saito, and K. Inui. Decreased activity and expression of intestinal oligopeptide transporter PEPT1 in rats with hyperthyroidism *in vivo*. *Pharm. Res.* **21**:969–975 (2004).
22. C. J. Torrance, J. E. Devente, J. P. Jones, and G. L. Dohm. Effects of thyroid hormone on GLUT4 glucose transporter gene expression and NIDDM in rats. *Endocrinology* **138**:1204–1214 (1997).
23. M. Matosin-Matekalo, J. E. Mesonero, T. J. Laroche, M. Lacasa, and E. Brot-Laroche. Glucose and thyroid hormone co-regulate the expression of the intestinal fructose transporter GLUT5. *Biochem. J.* **339**:233–239 (1999).
24. J. Huuskonen, M. Vishnu, C. R. Pullinger, P. E. Fielding, and C. J. Fielding. Regulation of ATP-binding cassette transporter A1 transcription by thyroid hormone receptor. *Biochemistry* **43**: 1626–1632 (2004).
25. R. A. Giannella, J. Orlowski, M. L. Jump, and J. B. Lingrel. Na^+-K^+-ATPase gene expression in rat intestine and Caco-2 cells: response to thyroid hormone. *Am. J. Physiol.* **265**:G775–782 (1993).
26. X. Li, A. J. Misik, C. V. Rieder, R. J. Solaro, A. Lowen, and L. Fliegel. Thyroid hormone receptor α_1 regulates expression of the Na^+/H^+ exchanger (NHE1). *J. Biol. Chem.* **277**:28656–28662 (2002).
27. P. O'Connor and J. Feely. Clinical pharmacokinetics and endocrine disorders. Therapeutic implications. *Clin. Pharmacokinet.* **13**:345–364 (1987).
28. H. R. Ochs, D. J. Greenblatt, H. J. Kaschell, U. Klehr, M. Divoll, and D. R. Abernethy. Diazepam kinetics in patients with renal insufficiency or hyperthyroidism. *Br. J. Clin. Pharmacol.* **12**:829–832 (1981).
29. M. Jin, T. Shimada, M. Shintani, K. Yokogawa, M. Nomura, and K. Miyamoto. Long-term levothyroxine treatment decreases the oral bioavailability of cyclosporin A by inducing P-glycoprotein in small intestine. *Drug Metab. Pharmacokinet.* **20**:324–330 (2005).
30. T. Mitin, L. L. von Moltke, M. H. Court, and D. J. Greenblatt. Levothyroxine up-regulates P-glycoprotein independent of the pregnane X receptor. *Drug Metab. Dispos.* **32**:779–782 (2004).