

Gastrectomy Increases the Expression of Hepatic Cytochrome P450 3A by Increasing Lithocholic Acid-Producing Enteric Bacteria in Mice

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We had previously revealed that drug metabolism, as well as the expression level of hepatic CYP3A, a drug-metabolizing enzyme, increase 12 weeks after gastrectomy in mice. In this study, we elucidated the mechanism of the increased CYP3A expression. The levels of lithocholic acid (LCA)-producing bacteria (*Bacteroides fragilis*) and LCA in the colon did not show a significant increase up to 4 weeks after gastrectomy compared to the sham operation group. In contrast, at 12 and 24 weeks post-gastrectomy, the levels of *Bacteroides fragilis* and LCA were significantly higher in the gastrectomy group than in the sham operation group. At 12 and 24 weeks after gastrectomy, the hepatic nuclear translocation of pregnane X receptor (PXR) had also increased. The hepatic CYP3A11 mRNA expression and nuclear translocation of PXR after intraperitoneal administration of LCA to normal mice was significantly higher than those of the control group. The intraperitoneal administration of taurolithocholic acid (TLCA), a taurine conjugate of LCA, caused no change in the expression level of CYP3A11. We suggest that the increase in the expression level of CYP3A after gastrectomy is caused by an increase in the nuclear translocation of PXR, which is triggered by an increase in LCA-producing bacteria.

Key words cytochrome P450; gastrectomy; lithocholic acid; pregnane X receptor

Gastric cancer is the second leading cause of cancer death worldwide and claims the lives of approximately 700000 people each year.^{1,2)} Surgical resection is the treatment of choice for patients with early stage gastric cancer.³⁾ Although there are complications such as dumping syndrome,⁴⁾ the malabsorption of lipids and proteins,⁵⁾ and anemia caused by the malabsorption of vitamin B₁₂ in patients with gastrectomy,⁶⁾ these complications can be treated effectively.

Immediately after gastrectomy, patients receive anticancer drugs such as tegafur and etoposide to prevent the postoperative recurrence of cancer, while antimicrobial drugs such as cefazolin and vancomycin are administered to prevent postoperative infection. Once the patients recover well enough to resume normal life, they may take various types of medications for various diseases, as in the case of the general public. Regarding drug therapy in patients with gastrectomy, it is known that the rate and pattern of drug absorption from the small intestine are different from those in healthy subjects due to the decrease in the gastric emptying rate (GER). For example, it has been reported that, after the administration of the analgesic agent acetaminophen to patients with gastric resections, a sharp increase in the blood concentration of acetaminophen is observed due to the shortened GER.⁷⁾ Because it is well known that the efficacy and side effects of drugs increases in patients with gastrectomy due to their shortened GER, proper measures must be taken in clinical practice when prescribing medications. However, other than the results mentioned above, little is known regarding drug therapy in patients with gastrectomy.

To fully elucidate the mechanism of the change in pharmacokinetics after gastrectomy, we recently conducted various experiments on CYP3A, the most important drug-metaboliz-

ing enzyme, using gastrectomy model mice.⁸⁾ In this study, we found that up to 4 weeks after gastrectomy, no changes in the hepatic expression of CYP3A or metabolic activity occurred. However, after 12 weeks, these values had increased significantly, and these levels were maintained for up to 24 weeks. The metabolism of an anti-cancer drug, imatinib, a substrate of CYP3A, accelerated as a result of the hepatic expression of CYP3A and of the increase in metabolic activity. However, the exact mechanism of the increase in the hepatic expression of CYP3A after gastrectomy remained unclear.

We have found that enteric bacteria are deeply involved in CYP3A. We have revealed that when the amount of lithocholic acid (LCA)-producing bacteria, such as *Bacteroides fragilis*, increased, the hepatic expression of CYP3A increased,^{9–11)} resulting in the decreased efficacy of drugs. It is known that the enterobacterial flora changes with the decrease in or lack of secretion of gastric acid in patients who have received a gastrectomy.¹²⁾ In particular, the levels of LCA-producing bacteria, which are known to thrive under alkaline conditions, increased.¹³⁾

We therefore hypothesized and attempted to verify that the following reasons may increase the hepatic expression level of CYP3A upon gastrectomy. In patients with gastrectomy, the level of LCA in the large intestine increased due to the increase in the pH of the gastrointestinal tract, which in turn causes the increase in the level of LCA-producing bacteria. Because the LCA produced in the colon reaches the liver via the portal vein, the level of LCA in the liver increases. Over 90% of the LCA that reaches the liver is immediately converted to taurine conjugate or glycine conjugate by bile acyl-CoA synthetase or bile acid-CoA : amino acid *N*-acyltransferase, respectively.¹⁴⁾ In the liver, LCA activates the nuclear receptor pregnane X receptor (PXR) and constitutive androstane receptor (CAR), eventually leading to an increase in the expression level of CYP3A.^{15,16)} Because CYP3A is a major drug-

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metabolizing enzyme, there is a possibility that the increase in CYP3A may enhance the metabolism of various drugs, leading to a decrease in the efficacy of the drugs.

In this study, we prepared mice with total gastrectomy and measured the expression level of CYP3A and the production level of LCA to evaluate the relationship between them. In addition, the mechanism of changes in the expression of CYP3A was revealed with a focus on LCA.

MATERIALS AND METHODS

Materials Bovine serum albumin (BSA), ethylenediamine-tetraacetic acid (EDTA), and TRI reagent were purchased from Sigma-Aldrich Corp. (St. Louis, MO, U.S.A.). Goat anti-mouse PXR antibodies, goat anti-mouse CAR antibodies, and donkey anti-goat immunoglobulin G-horseradish peroxidase (IgG-HRP) antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, U.S.A.). Enhanced chemiluminescence system (ECL) plus Western blotting detection reagents were purchased from GE Healthcare (Chalfont St. Giles, U.K.). The NE-PER nuclear extraction kit was purchased from Thermo Fisher Scientific (Waltham, MA, U.S.A.). A high-capacity cDNA synthesis kit was purchased from Applied Biosystems (Foster City, CA, U.S.A.), and iQ SYBR green supermix was purchased from Bio-Rad Laboratories (Hercules, CA, U.S.A.). Primers were purchased from Invitrogen Corp. (Tokyo, Japan). The QIAamp DNA stool mini kit was purchased from Qiagen, Inc. (Valencia, CA, U.S.A.). All of the other reagents were of the highest commercially available grade.

Animals Male ICR mice (7 weeks old) were purchased from Japan SLC, Inc. (Shizuoka, Japan). Mice were kept at room temperature ($24 \pm 1^\circ\text{C}$) at $55 \pm 5\%$ humidity with a 12 h light cycle (artificial illumination: 08:00–20:00). The present study was conducted according to the Guiding Principles for the Care and Use of Laboratory Animals, as adopted by the Committee on Animal Research at Hoshi University.

Gastrectomy Mice were divided into the gastrectomy and sham operation groups. Pentobarbital sodium salt was intraperitoneally administered to the mice at a dose of 50 mg/kg. In the gastrectomy group, the entire stomach was removed, and the esophagus was anastomosed to the duodenum.¹⁷⁾ In the sham operation group, a laparotomy was performed for an equal amount of time as the gastrectomy (approximately 15 min). The mice were given free access to water starting one day after the operation and were fed starting two days post-operation. For the gastrectomy group and the sham operation group, 20 animals each were prepared, and five animals from each group were used for the experiment at 2, 4, 12, and 24 weeks after the operation.

Collection of Samples The mice were dissected under ethyl ether anesthesia at 2, 4, 12, and 24 weeks after the op-

eration. The liver, appendix, and colon were removed from each mouse. The liver was frozen in liquid nitrogen and kept at -80°C until sample preparation. All contents of the appendix and colon were collected and suspended in purified water prior to homogenization with a Physcotron homogenizer. The homogenates were freeze-dried and stored at -80°C .

Preparation of RNA from Tissue Samples RNA was extracted from the frozen livers using TRI reagent. The resulting solution was diluted 50-fold using Tris-EDTA buffer (TE buffer). The RNA purity and concentration ($\mu\text{g}/\text{mL}$) were calculated by measuring the absorbance at 260 and 280 nm using a U-2800 spectrophotometer (Hitachi High Technologies, Tokyo, Japan).

Real-Time Polymerase Chain Reaction (PCR) A high-capacity cDNA synthesis kit was used to synthesize cDNA from $1 \mu\text{g}$ RNA. TE buffer was used to dilute the cDNA 20-fold to prepare the cDNA TE buffer solution. Target gene expression was detected by real-time reverse transcription (RT)-PCR using the primers listed in Table 1. To each well of a 96-well PCR plate, $25 \mu\text{L}$ of iQ SYBR green Supermix, $3 \mu\text{L}$ of target gene forward primer ($5 \text{ pmol}/\mu\text{L}$), $3 \mu\text{L}$ of reverse primer ($5 \text{ pmol}/\mu\text{L}$), $4 \mu\text{L}$ of cDNA TE buffer solution, and $15 \mu\text{L}$ of RNase-free water were added. To measure the 18S ribosomal RNA (rRNA) housekeeping gene, $4 \mu\text{L}$ of cDNA TE buffer solution was prepared by diluting the above-mentioned solution 20-fold with TE buffer. Real-time RT-PCR was conducted at a denaturation temperature of 95°C for 15 s, an annealing temperature of 56°C for 30 s, and an elongation temperature of 72°C for 30 s. The amplification of fluorescence intensity was monitored using the MyiQ™ single-color real-time RT-PCR detection system (Bio-Rad Laboratories). The mRNA gene expression levels were normalized to 18S rRNA gene expression.

Nuclear Extraction Nuclear protein extracts were prepared to examine PXR and CAR nuclear translocation. Proteins were extracted according to the NE-PER nuclear extraction kit protocol.

Electrophoresis and Immunoblotting Protein concentrations were measured by the bicinchoninic acid (BCA) method using BSA as a standard.¹⁸⁾ Electrophoresis was performed using Laemmli's method.¹⁹⁾ Proteins were diluted twofold using loading buffer (84 mM Tris, 20% glycerol, 0.004% bromophenol blue, 4.6% sodium dodecyl sulfate (SDS), and 10% 2-mercaptoethanol, pH 6.8). Samples were boiled for 5 min prior to loading on a polyacrylamide gel. After electrophoresis, isolated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane, which was incubated in 1% skim milk blocking buffer for 1 h. After blocking, the membrane was incubated with primary antibodies for 1 h at room temperature. The following primary antibodies were used: goat anti-mouse PXR (1/1000) and rabbit anti-mouse CAR (1/1000). After washing the membrane with TBS-Tween (20 mM Tris-HCl, 137 mM NaCl, and 0.1% Tween 20, pH 7.6),

Table 1. Primer Sequences of Mouse mRNAs

Target	Accession number	Forward primer (5' to 3')	Reverse primer (5' to 3')
CYP3A11	NM_007818	CGCCTCTCCTTGCTGTCACA	CTTTCCTTCTGCCTCAAGT
CYP2B10	NM_009999	CTGTCGTTGAGCCAACCTTC	GGTGGCCAGAGAGAATCTAC
ABCC3	NM_029600	GGTACTCCTGCTCGAAGAC	CTTGCGGACCTCGTAGATG
18S rRNA	X00686	GTCTGTGATGCCCTTAGATG	AGCTTATGACCCGCACTTAC

the membrane was incubated with secondary antibodies for 1 h at room temperature. The following secondary antibodies were used: donkey anti-goat IgG-HRP antibody (1/1000) and donkey anti-rabbit IgG-HRP antibody (1/1000). After washing, the membrane was incubated with ECL Plus detection reagent and visualized with an LAS-3000 Mini Lumino image analyzer (FUJIFILM, Tokyo, Japan).

DNA Preparation from Large Intestine Content Samples

DNA was extracted from approximately 200 mg of frozen large intestine content using a QIAamp DNA stool mini kit. DNA extraction was performed according to the QIAamp DNA stool mini kit protocol.

Quantification of LCA-Producing Bacteria *Bacteroides fragilis* was detected using real-time PCR. Into each well of a PCR plate, 25 μ L of iQ SYBR Green Supermix, 3 μ L of *Bacteroides fragilis* forward primer (5 pmol/ μ L), 3 μ L of reverse primer (5 pmol/ μ L), 2 μ L of DNA TE buffer solution, and 17 μ L of RNase-free water were added. The following primer pair was used for *Bacteroides fragilis*: forward 5'-ctgaaccagc-ccaagtgcg-3' and reverse 5'-ccgcaaacttcacaactgactta-3'. The denaturation temperature was 95°C for 30 s, the annealing temperature was 58°C for 30 s, and the elongation temperature was 72°C for 1 min. The fluorescence intensity during amplification was monitored using the MyiQ™ single color real-time RT-PCR detection system.

Extraction of LCA in the Large Intestine Content A total of 15 mL of ethyl acetate was added to approximately 100 mg of freeze-dried large intestine content, and the mixture was sonicated to form a suspension. A total of 150 μ L of HCl solution was added to the suspension in order to obtain a non-ionic form of LCA, which was extracted into an ethyl acetate phase. The mixture was agitated for 2 h in a warm bath at 50°C. The suspension was centrifuged (12000 $\times g$ for 10 min at 4°C), and the supernatant was dried under nitrogen gas. The residue was dissolved in 1 mL acetonitrile and mixed with 25 μ L of Br-DMEQ solution, 25 μ L of 18-crown-6 solution, and 5 mg of K₂CO₃. The mixture was heated for 20 min at 80°C in the dark. After cooling, the mixture was centrifuged (13400 $\times g$ for 1 min at 25°C). The supernatant was used as an HPLC sample.²⁰⁾ With this method, TLCA is not hydrolyzed to LCA. Therefore, the original ratio of TLCA to LCA in the large intestine can be examined directly.

Quantification of LCA Using HPLC The HPLC apparatus consisted of a 600S controller (Waters, Tokyo, Japan), a 616 pump (Waters), a 717plus Autosampler (Waters), and a Waters 2475 λ Fluorescence Detector (Waters). Inertsil C18 ODS-3 was used as the column (with a mean particle size of 5 μ m, 4.6 \times 150 mm, GL Sciences Inc., Tokyo, Japan). A solution of water–acetonitrile–methanol (21:66:13) was used as the mobile phase. The flow rate was 2.0 mL/min, and the temperature was 40°C. The excitation wavelength was 370 nm, and the detection wavelength was 455 nm.²¹⁾

Treatment of LCA and TLCA for Normal Mice LCA (15 mg/kg) or tauroolithocholic acid (TLCA) (15 mg/kg) was intraperitoneally administered to 8-week-old normal mice. Two hours after the administration of LCA or TLCA, the expression level of CYP3A11 mRNA, LCA concentration, TLCA concentration, and the nuclear translocation of PXR and CAR in the liver were analyzed.

Extraction of LCA and TLCA from the Liver A total of 250 μ L purified water was added to approximately 75 mg

of liver and homogenized by 5 min of ultrasonic treatment, after which 1.5 mL of methanol was added to the homogenate. The mixture was heated at 85°C for 1 min before centrifugation (16100 $\times g$ for 5 min). In total, 1.5 mL of supernatant was filtered through a 0.45- μ m membrane filter. The filtrate was evaporated to dryness under nitrogen gas. The resulting residue was dissolved in 100 μ L of methanol for LC/MS analysis.²²⁾

Quantification of LCA and TLCA Using LC/MS A Waters 2695 Separations Module (Waters) and a 3100 Mass Detector (Waters) were used as the LC/MS system. An Imtakt Cadenza CD-C18 column (2.0 mm i.d. \times 30 mm, Imtakt Corporation, Kyoto, Japan), and a mobile phase consisting of A (100 mM AcONH₄–AcOH, pH 4.5), B (water), and C (acetonitrile) were used for HPLC separation. The gradient elution conditions for HPLC were as follows: B–C–A (80:10:10, 0 to 1 min hold) to 35:55:10 (10 min linear gradient) to 0:90:10 (60 min linear gradient) to 0:90:10 (65 min hold) to 80:10:10 (68 min linear gradient). The flow rate was 0.2 mL/min. Ionization was performed by electrospray ionization (ESI). Negative mode detection was conducted.²³⁾ The standard curves were generated using 0.026 nM, 0.26 nM, 2.6 nM, 26 nM, 260 nM, and 2.6 μ M of LCA and TLCA solutions in methanol. The retention time of LCA and TLCA were 55.5 min and 42.0 min, respectively, and the both lower limit of quantification were 0.053 nmol/g liver.

Statistical Analyses Numerical data were expressed as the means \pm standard deviation. Significance was examined using Student's *t*-test for pairs of values. Differences of *p*<0.05 were considered to be statistically significant.

RESULTS

The Expression Level of CYP3A11 mRNA and CYP3A Protein in the Liver Hepatic CYP3A11 mRNA and CYP3A protein expression levels were analyzed 2, 4, 12, and 24 weeks after gastrectomy (data not shown).⁸⁾

CYP3A11 of mice is highly homologous with CYP3A4 of humans. In addition, CYP3A11 is one of the CYP that are most abundantly expressed.²⁴⁾ Therefore, the analysis was performed by focusing on CYP3A11. No differences were observed in hepatic CYP3A11 mRNA and CYP3A protein expression levels between the gastrectomy and sham operation groups at 2 and 4 weeks post-operation. At 12 and 24 weeks post-operation, however, the expression levels of hepatic CYP3A11 mRNA and CYP3A protein were significantly higher (approximately three times higher) in the gastrectomy group than in the sham operation group at 12 and 24 weeks post-operation (data not shown).⁸⁾

The Level of LCA-Producing Bacteria and LCA in the Colon It has been revealed that LCA, the secondary bile acid that is produced by enteric bacteria, increases hepatic CYP3A expression levels by enhancing CYP3A transcription.²⁵⁾ Therefore, the mechanism by which the gastrectomy increased hepatic CYP3A expression levels was examined with a focus on LCA. LCA-producing bacteria (*Bacteroides fragilis*) and LCA levels in the colon were examined from 2 to 24 weeks post-operation (Fig. 1).

No differences were observed in *Bacteroides fragilis* levels at 2 and 4 weeks post-operation between the two groups. However, *Bacteroides fragilis* levels were significantly higher

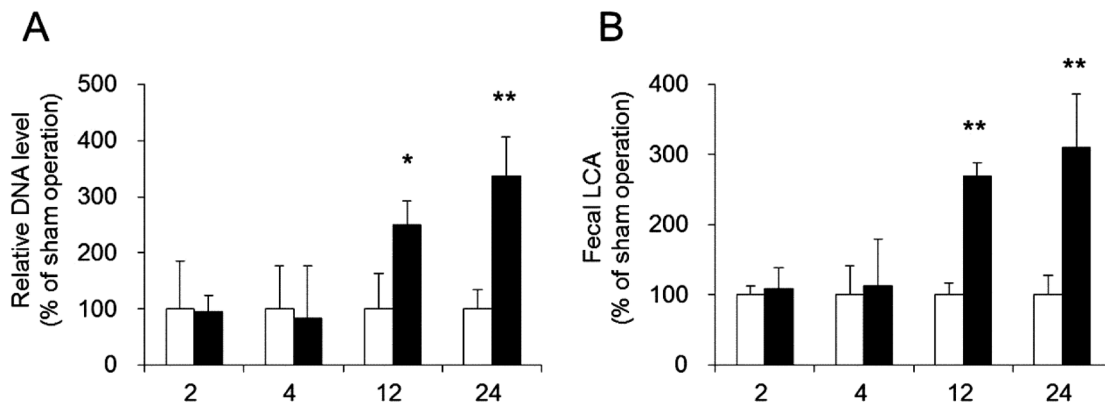


Fig. 1. The Levels of LCA-Producing Bacteria and LCA in the Colon

The mice in the sham operation group (white) and the gastrectomy group (black) were allowed free access to the diets for 2, 4, 12, or 24 weeks. The amount of *Bacteroides fragilis* DNA found in the colon was measured using real-time PCR (A). LCA was extracted from the large intestine content, and the concentration of LCA was measured using HPLC (B). Data are presented as the means \pm S.D. and were obtained from five mice per group. Student's *t*-test; * p <0.05 and ** p <0.01 vs. the sham operation group.

(approximately three times higher) in the gastrectomy group compared with the sham operation group at 12 and 24 weeks (Fig. 1A).

No differences were observed in the LCA levels in the colon at 2 and 4 weeks post-operation between the gastrectomy and sham operation groups. However, LCA levels in the gastrectomy group were significantly higher than in the sham operation group at 12 and 24 weeks after the operation (Fig. 1B).

Because the changes in LCA-producing bacteria and LCA levels in the colon were consistent with the changes in the hepatic CYP3A expression levels, it was hypothesized that the enteric bacteria-mediated increase in LCA levels was involved in the increased CYP3A expression levels that were observed after the gastrectomy.

LCA and TLCA Levels in the Liver We examined whether the LCA that was produced by *Bacteroides fragilis* in the large intestine reached the liver. Thus, hepatic LCA and its conjugate, TLCA, were measured by LC/MS at 12 weeks post-gastrectomy, when increased colonic LCA levels were observed, and these results were compared with those of the sham operation group (Fig. 2).

The hepatic LCA concentration in the gastrectomy group and the sham operation group were lower than the levels that could be detected by LC/MS (Fig. 2A). On the other hand, it was confirmed that the hepatic concentration of TLCA was significantly higher in the gastrectomy group than in the sham operation group (Fig. 2B).

As mentioned above, the increase in the hepatic LCA concentration could not be directly verified in this study. However, the results of this study suggest that there is a possibility that the LCA that reached the liver increased but was immediately converted to TLCA once it reached the liver.

Effect of LCA or TLCA on CYP3A Expression Levels Based on the above-mentioned experiments, there is a possibility that gastrectomy causes an increase in hepatic LCA that is immediately converted to TLCA. Therefore, we examined whether the increase in hepatic CYP3A expression levels after gastrectomy was attributable to the effect of LCA or TLCA. The mRNA expression level of CYP3A11 in the liver was analyzed after the intraperitoneal administration of LCA or TLCA to normal mice (Fig. 3).

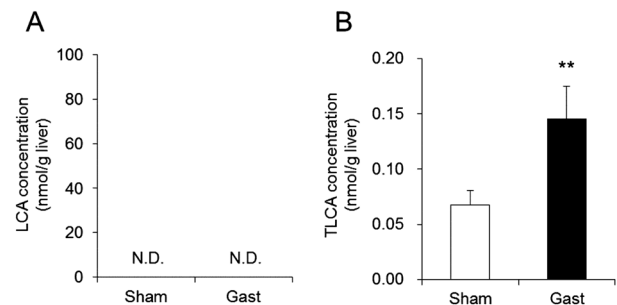


Fig. 2. The Level of LCA and TLCA in the Liver

The mice in the sham operation group and the gastrectomy group were allowed free access to the diets for 12 weeks. LCA and TLCA were extracted from the liver, and the concentrations of LCA (A) and TLCA (B) were measured using LC/MS. Data are presented as the means \pm S.D. and were obtained from five mice per group. Student's *t*-test; ** p <0.01 vs. the sham operation group.

The mRNA expression level analysis of hepatic CYP3A11 at 2 h after the administration of LCA revealed that the expression level of CYP3A11 increased approximately two times compared to the level immediately after the administration of LCA (Fig. 3A). At this time point, LCA was detected in the liver and the TLCA concentration was increasing (Fig. 3B). In addition, based on the examination of the nuclear translocation level of PXR and CAR in the liver, it was revealed that the nuclear translocation level of PXR was increased by LCA administration, while no changes were observed in the nuclear translocation level of CAR (Figs. 3C, D). In the livers of mice, to which the TLCA was intraperitoneally administered, an increase in the mRNA expression level of CYP3A11 was not observed, even at 2 h after administration (Fig. 3E). At this time point, LCA was not detected in the liver; however, the TLCA concentration was increasing (Fig. 3F). In addition, based on the examination of the nuclear translocation level of PXR and CAR in the liver, it was revealed that the nuclear translocation levels of PXR and CAR were not changed by TLCA administration (Figs. 3G, H).

These results strongly suggest that LCA is involved in an increase in the CYP3A expression level after gastrectomy, whereas TLCA had little effect on the increased CYP3A expression level in the liver.

The Nuclear Translocation of PXR and the ABCC3 mRNA Expression Level in the Liver LCA binding to

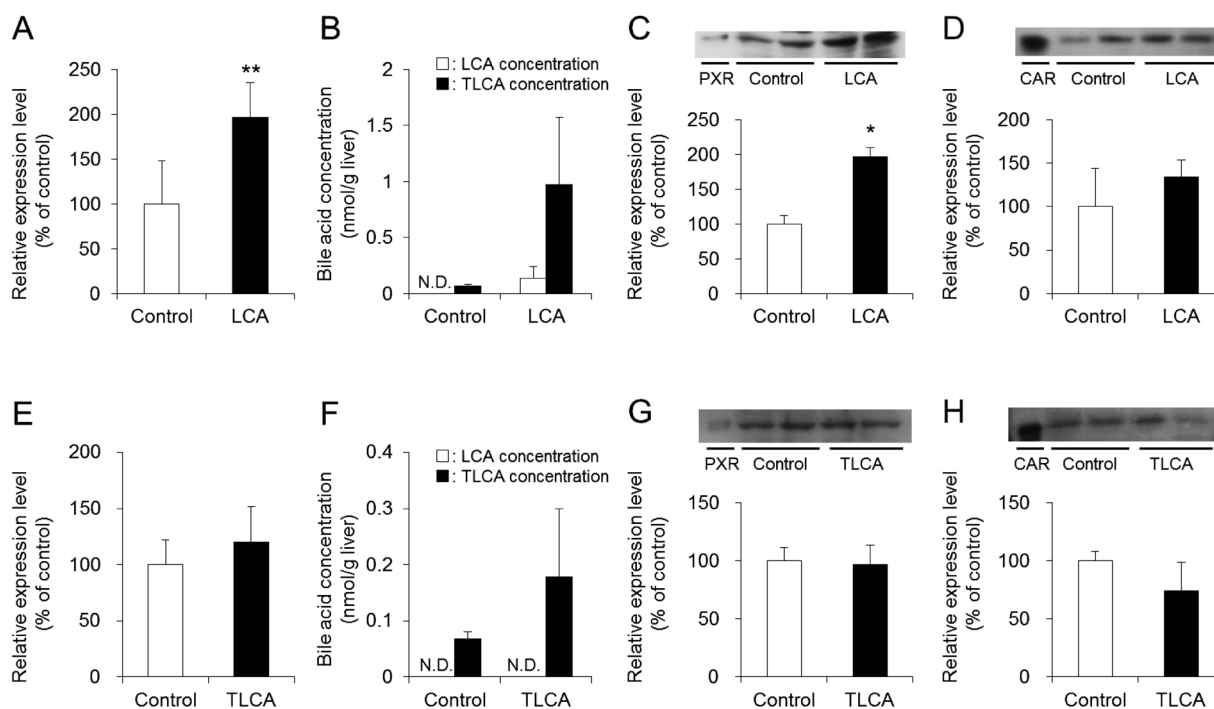


Fig. 3. Effect of LCA or TLCA on the CYP3A Expression Level

The liver was removed 2h after intraperitoneal LCA (A–D) or TLCA (E–H) administration to normal mice. Hepatic CYP3A11 mRNA expression levels were measured by performing real-time RT-PCR and were normalized to 18S rRNA (A and E). LCA and TLCA were extracted from the liver, and LCA and TLCA concentrations were measured using LC/MS (B and F). Nuclear fractions were prepared from the livers of mice after intraperitoneal LCA or TLCA administration, and the expression levels of the PXR (C and G) and CAR (D and H) protein were measured by performing Western blotting analysis. The results are presented by setting the mean of the control group at 100%. The data are presented as the means \pm S.D. and were obtained from five mice per group. Student's *t*-test; * p < 0.05 and ** p < 0.01 vs. the control group.

PXR resulted in enhanced CYP3A11 transcription and protein expression levels.²⁵⁾ We examined whether the increase in CYP3A after gastrectomy was attributable to an increase in PXR nuclear translocation. Hepatic nuclear fractions were prepared, and PXR protein expression levels were examined by Western blot. The expression of ATP-binding cassette, sub-family C (MRP), member 3 (ABCC3) is also regulated by PXR. Therefore, when the expression of CYP3A11 is regulated by PXR, the expression of not only CYP3A11 but also ABCC3 is expected to increase. In this study, the expression of ABCC3 was also examined²⁶⁾ (Fig. 4).

Nuclear PXR levels 2 and 4 weeks after the gastrectomy were nearly identical to those in the sham operation group. Meanwhile, nuclear PXR levels were significantly higher 12 and 24 weeks after the gastrectomy than in the sham operation group (Fig. 4A). ABCC3 mRNA expression levels 2 and 4 weeks after gastrectomy were similar to those in the sham operation group. However, ABCC3 mRNA expression levels were significantly higher in the gastrectomy group than in the sham operation group at 12 and 24 weeks post-gastrectomy (Fig. 4B). ABCC3 mRNA levels followed a similar pattern to that of CYP3A11 mRNA and PXR nuclear translocation.

These results demonstrated that gastrectomy increases hepatic nuclear PXR, which enhances CYP3A11 and ABCC3 transcription.

The Nuclear Translocation of CAR and the CYP2B10 mRNA Expression Level in the Liver CYP3A11 transcription is regulated not only by PXR but also by the nuclear receptor CAR.²⁷⁾ Therefore, we examined whether nuclear CAR levels increased by the gastrectomy. Hepatic nuclear fractions were prepared, and CAR protein expression levels were examined by Western blot to determine the level of nuclear

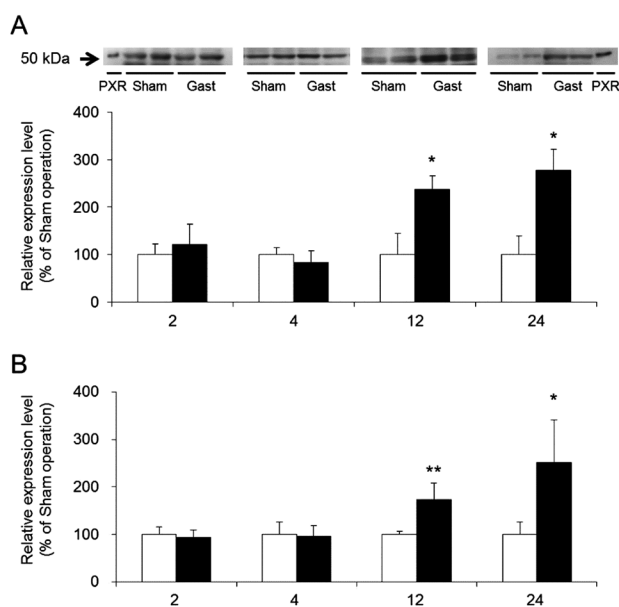


Fig. 4. The Nuclear Translocation of PXR and the ABCC3 mRNA Expression Level in the Liver

The mice of the sham operation group (white) and the gastrectomy group (black) were allowed free access to the diet. Nuclear fractions were prepared from the livers of mice at 2, 4, 12, or 24 weeks after the operation, and the expression levels of the PXR protein were measured by performing Western blotting analysis (A). ABCC3 mRNA expression levels in the liver were measured by performing real-time RT-PCR and were normalized to 18S rRNA (B). The results are presented by setting the mean of the sham operation group at 100%. Data are presented as the means \pm S.D. and were obtained from five mice per group. Student's *t*-test; * p < 0.05 and ** p < 0.01 vs. the sham operation group.

CAR. The expression of CYP2B10 is also regulated by CAR. Therefore, when the expression of CYP3A11 is regulated by CAR, the expression of not only CYP3A11 but also CYP2B10 is expected to increase. The expression of CYP2B10 was also examined²⁷⁾ (Fig. 5).

The nuclear CAR levels 2 and 4 weeks after the gastrectomy were similar in the two operation groups. However, the nuclear CAR levels 12 and 24 weeks after the gastrectomy were significantly higher than those in the sham operation group (Fig. 5A). CYP2B10 mRNA expression levels at 2 and 4 weeks after gastrectomy were similar to those of the sham operation group. However, 12 and 24 weeks after gastrectomy, the CYP2B10 mRNA expression levels were significantly higher in the gastrectomy group than in the sham operation group (Fig. 5B). The pattern of CYP2B10 mRNA levels was similar to that of the CYP3A11 mRNA levels and CAR nuclear translocations.

These results demonstrate that gastrectomy increases hepatic CAR nuclear translocation, thus enhancing CYP3A11 and CYP2B10 transcription.

DISCUSSION

In the past, whether or not the medicines were properly used in the patients with gastrectomy was not investigated. In this study, focusing on drug-metabolizing enzyme CYP, we examined the changes in CYP expression from 2 to 24 weeks after the gastrectomy. The reasons why we chose 24 weeks after the gastrectomy as the endpoint were as follows. The average age at the onset of gastric cancer in humans is approx. 60 years old. Suppose patients undergo gastrectomy at the age of 60, then they are likely to live another 20 years, based on the average life expectancy of humans (approx. 80 years). Twenty years for humans correspond to approximately 24 weeks for mice, based on the life expectancy of mice (approx. 2 years=96 weeks). Based on the above, we chose 24 weeks as the endpoint in this study. We have revealed that no changes in the hepatic expression of CYP3A occurred up to 4 weeks after gastrectomy; however, CYP3A expression began to increase after 12 weeks.⁸⁾ In this study, we analyzed the mechanism of the increased hepatic CYP3A expression.

Previous research has confirmed the following. The pH in the gastrointestinal tract of patients with gastrectomy increases due to a decrease in or lack of gastric acid secretion.¹²⁾ In the intestinal environment, where the pH is high, the level of enteric bacteria with an elevated activity of the enzyme 7 α -dehydroxylase, which converts chenodeoxycholic acid to LCA,¹³⁾ increases. LCA activates hepatic nuclear receptors, such as PXR or CAR, which cause an increase in the expression level of hepatic CYP3A.^{16,25)} *Bacteroides fragilis* and LCA levels were significantly higher in the gastrectomy group than in the sham operation group (Fig. 1). The patterns of LCA-producing bacteria and LCA levels were similar to that of the CYP3A levels. It was, therefore, suggested that the enterobacterial flora-mediated increase in LCA levels might be involved in the increased hepatic CYP3A expression levels that were observed after gastrectomy. On the other hand, the level of *Bacteroides fragilis* did not increase up to 4 weeks after the gastrectomy, but began to increase from 12 weeks after the gastrectomy onward. Therefore, it was considered that a certain period of time is required to establish stable

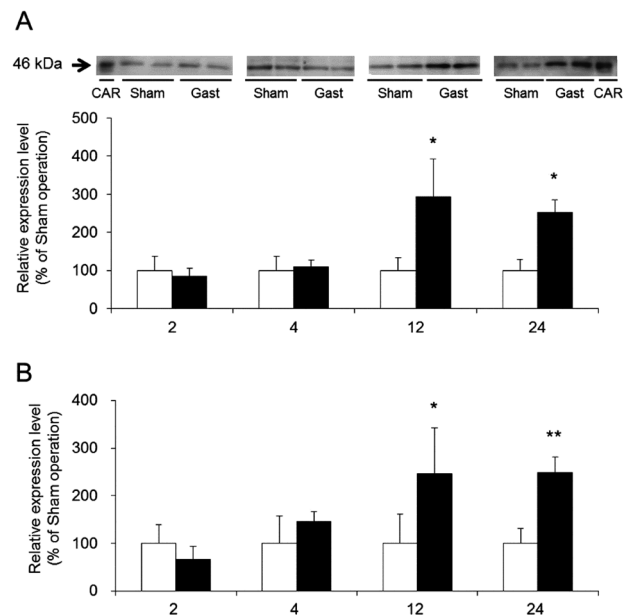


Fig. 5. The Nuclear Translocation of CAR and the CYP2B10 mRNA Expression Levels in the Liver

The mice of the sham operation group (white) and the gastrectomy group (black) were allowed free access to the diets. Nuclear fractions were prepared from the livers of mice at 2, 4, 12, or 24 weeks after the operation, and the expression level of the CAR protein was measured by performing Western blotting analysis (A). CYP2B10 mRNA expression levels in the liver were measured by performing real-time RT-PCR and were normalized to 18S rRNA (B). The results are presented by setting the mean of the sham operation group at 100%. The data are presented as the means \pm S.D. and were obtained from five mice per group. Student's *t*-test; * p <0.05 and ** p <0.01 vs. the sham operation group.

enterobacterial flora after the gastrectomy. In addition, it is known that *Bacteroides fragilis* possesses activity of converting the glycine conjugate of LCA to LCA, along with the activity of deconjugation of TLCA to LCA.²⁸⁾ Therefore, it was considered that the conversion from amino acid conjugate of LCA to LCA was enhanced by the increase in *Bacteroides fragilis*.

We then examined whether hepatic LCA levels increased in the gastrectomy group. As a result, although LCA was not detected in the liver²⁹⁾ (Fig. 2A), high levels of TLCA, which is a taurine conjugate of LCA, were detected in the livers of the gastrectomy group (Fig. 2B). Therefore, it was necessary to verify whether the increase in CYP3A expression in the gastrectomy group was attributable to the effect of LCA or TLCA. When LCA was intraperitoneally administered to normal mice, twice increase in CYP3A expression was observed (Fig. 3A). This increase in CYP3A after the intraperitoneal administration of LCA was approximately 30% lower than the CYP3A increase observed from 12 to 24 weeks after the gastrectomy.⁸⁾ On the other hand, when TLCA was intraperitoneally administered to the gastrectomy mice, an increase in CYP3A expression was not observed (Fig. 3E). Even when a high dose of TLCA (125 mg/kg)^{15,30)} was intraperitoneally administered to the normal mice, an increase in CYP3A expression was still not observed (data not shown). In the experiment of intraperitoneal administration of LCA or TLCA to normal mice, an increase in CYP3A was observed and LCA was detected in the liver only after the administration of LCA. In this study, these concentrations were measured at 2h after the administration of LCA and TLCA. The reason why this time point of measurement was selected is that it was known that

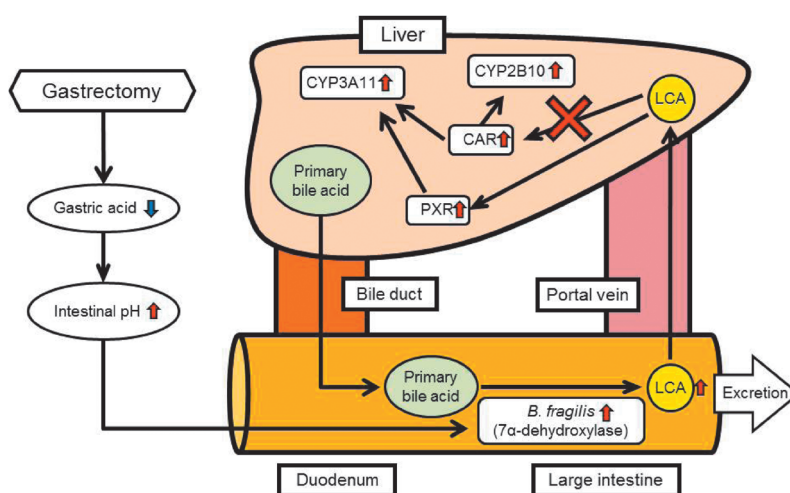


Fig. 6. Mechanism of Changes in CYP Expression Caused by Gastrectomy

In patients with gastrectomy, the level of LCA in the large intestine increased due to the increase in the pH of the gastrointestinal tract, which in turn causes the increase in the level of LCA-producing bacteria (*Bacteroides fragilis*). The LCA produced in the colon reaches the liver via the portal vein. In the liver, LCA activates the nuclear PXR, eventually leading to an increase in the expression level of CYP3A. On the other hand, LCA was not involved in the increase in nuclear translocation level of CAR.

LCA is immediately converted to TLCA in the liver.²⁹⁾ In addition, to evaluate the effect of LCA in this study, the amount of LCA that was intraperitoneally administered was higher than that observed under physiological conditions. It was considered that the reason why LCA was not detected in the liver of gastrectomy mice, but that it was detected in the liver of mice to which LCA was intraperitoneally administered, is that the LCA was immediately converted to TLCA, and that there was a difference in the administered amount of LCA between these two groups. Based on these results, it was strongly suggested that, although LCA was immediately converted to TLCA, LCA was still involved in the increase in CYP3A due to its function as a PXR ligand.

When LCA binds to the nuclear receptor PXR, the nuclear translocation of PXR occurs, and CYP3A transcription is enhanced.^{25,30)} Nuclear PXR levels were therefore examined. The results indicated that 12 and 24 weeks after gastrectomy, when increased CYP3A expression levels were observed, hepatic nuclear PXR translocation significantly increased in the gastrectomy group, compared with the sham operation group (Fig. 4A). The pattern of variation in the ABCC3 mRNA expression levels, the transcription of which is regulated by PXR,²⁶⁾ was similar to that of CYP3A (Fig. 4B). When LCA was intraperitoneally administered to normal mice, an increase in the CYP3A expression level in the liver was observed, along with an increase in the nuclear translocation level of PXR (Fig. 3). Based on the above-mentioned results, it is possible that gastrectomy may cause an increase in the level of LCA-producing bacteria, resulting in an increase in the LCA production level and an enhanced nuclear translocation of PXR in the liver, which eventually lead to an increase in the expression level of CYP3A (Fig. 6). CAR is not only involved in the expression of CYP3A, but also in the expression of other CYPs such as CYP2B.²⁷⁾ The increase in the nuclear translocation level of CAR in the gastrectomy mice was consistent with the increase in the expression of CYP3A11 and CYP2B10 (Fig. 5B). Based on these results, it was considered that the increases in nuclear translocation level, not only of PXR but also CAR, were involved in the increase in the mRNA expression level of CYP3A11. However, no changes

were observed in the nuclear translocation level of CAR after the intraperitoneal administration of LCA to normal mice (Fig. 3D). Based on these results, it was considered that LCA was not involved in the increase in nuclear translocation level of CAR from 12 to 24 weeks after gastrectomy. This may be one of the reasons why the increase in CYP3A expression in the gastrectomy group was 3-fold,⁸⁾ while that after the intraperitoneal administration of LCA was 2-fold (Fig. 3A). It was reported that the administration of LCA to wild type mice resulted in an increase in hepatic CYP3A expression level, while LCA administration to PXR knockout mice caused no changes in hepatic CYP3A expression level.¹⁵⁾ Based on this, it was considered that the nuclear translocation of PXR played an important role in the increase in the CYP3A expression level caused by LCA.

Regarding drug therapies in patients with gastrectomy, past attention has only been paid to the effect of a shortened GER. However, the results of this study have provided the following new knowledge regarding drug therapy in patients with gastrectomy. In patients with gastrectomy, the activity of CYPs specifically increased in the liver. Therefore, there is a possibility that the effects of the drug, which is mainly metabolized by hepatic CYPs, may be decreased after both oral and non-oral administrations. This increase in CYP activity is not observed immediately post-operation but rather begins when the LCA-producing bacteria are colonized, at which point it may persist for life. In addition, because the increase in CYP activity in patients with gastrectomy is attributable to the increase in LCA-producing bacteria, care should be taken when using drugs that may affect the LCA-producing bacteria.

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