**Table 1** Characteristics of HBV carriers according to hepatologist consultation (n＝126)

| | with consultation n＝26 | without consultation n＝100 | *P*-value |
|---|---|---|---|
| Age (years) | 50 (19-78) | 64 (16-91) | 0.003 |
| Males/females | 13/13 | 43/57 | 0.658 |
| Department: Internal Medicine/others | 17/9 | 34/66 | 0.006 |
| Platelet (×10⁴/μL) | 20.2 (3.2-36.4) | 20.1 (2.9-66.5) | 0.545 |
| AST (IU/L) | 25 (12-450) | 24 (9-558) | 0.598 |
| ALT (IU/L) | 22 (9-93) | 19 (5-189) | 0.657 |
| AST/ALT ratio | 1.26 (0.57-4.84) | 1.23 (0.40-5.58) | 0.638 |
| γ-GTP (IU/L) | 17 (7-998)† | 22 (8-1,590)‡ | 0.737 |
| HBsAg (IU/mL) | 1698 (0.07-21,792) | 188 (0.07-106,400) | 0.190 |
| HBeAg positive/negative | 2/17 | 7/61 | 1.000 |
| with/without hepatitis | 13/13 | 53/47 | 0.828 |

Values are median (range) or number of patients. †n＝25, ‡n＝85.

**Table 2** Univariate and multivariate analysis of factors associated with hepatologist consultation for HBV carriers (n＝126)

| Factors | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value |
| Age | 0.963 (0.937-0.989) | 0.006 | 0.965 (0.938-0.992) | 0.012 |
| Sex: male | 1.326 (0.558-3.148) | 0.523 | | |
| Department: except Internal Medicine | 0.273 (0.110-0.676) | 0.005 | 0.294 (0.115-0.748) | 0.010 |
| Platelet (×10⁴/μL) | 1.007 (0.958-1.058) | 0.784 | | |
| AST (IU/L) | 1.003 (0.997-1.008) | 0.370 | | |
| ALT (IU/L) | 1.003 (0.988-1.019) | 0.688 | | |
| γ-GTP (IU/L)† | 1.000 (0.998-1.002) | 0.806 | | |
| HBsAg (IU/mL) | 1.000 (1.000-1.000) | 0.525 | | |
| HBeAg‡: positive | 1.025 (0.195-5.397) | 0.977 | | |

†n＝110, ‡n＝87.

HBVキャリア126例のうち介入群は26例（21％），非介入群は100例（79％）であった（Fig. 1a）．2群間の比較では，非介入群は有意に高齢であり，非内科系診療科が有意に多かった（Table 1）．また，コンサルトに寄与する因子の多変量解析でも，年齢（odds ratio [OR]：0.965，95% confidence interval [CI]：0.938–0.992, $p$＝0.012）と診療科（非内科系診療科，OR：0.294，95％CI：0.115–0.748，$p$＝0.010）が関与していた（Table 2）．推定B型慢性肝炎66例のうちコンサルトに寄与する因子の単変量解析では，診療科（非内科系診療科，OR：0.269，95％CI：0.073–0.991，$p$＝0.048）が唯一関与しており，多変量解析では診療科（非内科系診療科，OR：0.270，95％CI：0.072–1.017，$p$＝0.053）で傾向を認めた（Table 3）．肝炎のないHBVキャリア60例のうちコンサルトに寄与する因子の単変量解析では，年齢（OR：0.958，95％CI：0.923–0.993，$p$＝0.02）と診療科（非内科系診療科，OR：0.265，95％CI：0.074–0.954，$p$＝0.042）が関与しており，多変量解析では年齢（OR：961，95％CI：0.926–0.998，$p$＝0.039）が唯一関与していた．

非介入群の診療内容調査では，50例はHBVについて全く記載がなく，9例は既往歴に記載があるのみでプロブレムリストには挙げられておらず，30例はプロブレムリストに挙げられているものの診療方針が記載されていなかった（Table 4）．

**Table 3** Univariate and multivariate analysis of factors associated with hepatologist consultation for probable chronic hepatitis B individuals (n＝66)

| Factors | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value |
| Age | 0.969 (0.930-1.009) | 0.125 | 0.969 (0.929-1.010) | 0.140 |
| Sex: male | 1.227 (0.354-4.250) | 0.747 | | |
| Department: except Internal Medicine | 0.269 (0.073-0.991) | 0.048 | 0.270 (0.072-1.017) | 0.053 |
| Platelet ($\times 10^4/\mu L$) | 0.988 (0.927-1.054) | 0.724 | | |
| AST (IU/L) | 1.003 (0.997-1.009) | 0.292 | | |
| ALT (IU/L) | 1.006 (0.989-1.024) | 0.482 | | |
| γ-GTP (IU/L)† | 1.000 (0.998-1.002) | 0.785 | | |
| HBsAg (IU/mL) | 1.000 (1.000-1.000) | 0.491 | | |
| HBeAg‡: positive | 1.667 (0.151-18.455) | 0.677 | | |

†n＝60, ‡n＝46.

**Table 4** Situation of medical practice for HBV carriers without hepatologist consultation (n＝100)

| Descriptions regarding HBV on electronic medical record | n (%) |
|---|---|
| nothing | 50 (50) |
| written in the past history, but not listed on the problem lists | 9 (9) |
| listed on the problem lists, but not mentioned in the plan for medical practice | 30 (30) |
| listed on the problem lists and mentioned in the plan for medical practice | 11 (11) |

**b) Anti-HCV 検査**

Anti-HCV 測定者 6,612 例のうち，陽性者は 487 例 (7.4%)，そのうち低力価 118 例，中力価 114 例，高力価 255 例であり，推定 HCV キャリアは 369 例 (5.6%) であった．そのうち血小板数$<15\times10^4/\mu L$, AST≦ALT, ALT≧31 IU/L を満たすものはそれぞれ 151 例，97 例，139 例であり，推定 C 型慢性肝炎は 244 例 (3.7%) であった (Fig. 1b)．27 診療科のうち測定数の多い上位 10 科は，整形外科 875 例，眼科 728 例，産婦人科 531 例，一般・消化器外科 519 例，循環器内科 402 例，救急科 334 例，消化器内科 286 例，総合診療科 277 例，泌尿器科 268 例，歯科口腔外科 263 例であった (Fig. 3a)．推定 HCV キャリア数は，整形外科 52 例，一般・消化器外科 50 例，眼科 47 例，消化器内科 25 例，循環器内科 25 例，救急科 22 例，総合診療科 15 例，腎臓内科 13 例，皮膚科 12 例，歯科口腔外科 12 例の順で多く，これら上位 10 科のうち肝臓内科へのコンサルト率は消化器内科，総合診療科，一般・消化器外科，救急科で比較的高い傾向がみられたものの 3 割前後に留まっていた (Fig. 3b)．陽性率では，腎臓内科 10.7%，一般・消化器外科 9.6%，消化器内科 8.7%，心臓血管外科 7.2%，救急科 6.6%，眼科 6.5%，呼吸器内科 6.3%，循環器内科 6.2%，脳神経外科 6.0%，整形外科 5.9% の順で高率であった．

推定 HCV キャリア 369 例のうち介入群は 67 例 (18%)，非介入群は 302 例 (82%) であった (Fig. 1b)．両群間の比較では，非介入群では血小板数が有意に多く，AST 値，ALT 値，γ-GTP 値，Anti-HCV 抗体価が有意に低値であった (Table 5)．また，コンサルトに寄与する因子の多変量解析では，ALT 値 (OR：1.014，95%CI：1.004–1.024，$p=0.007$) と Anti-HCV (OR：1.196，95% CI：1.073–1.334，$p=0.001$) が関与していた (Table 6)．推定 C 型慢性肝炎 244 例のうちコンサルトに寄与する因子の多変量解析では，血小板数 (OR：0.897, 95%CI：0.831–0.968，$p=0.005$)，ALT 値 (OR：1.020，95%CI：1.008–1.033，$p=0.001$)，Anti-HCV (OR：1.283，95% CI：1.085–1.518，$p=0.004$) が関与していた (Table 7)．肝炎のない推定 HCV キャリア 125 例のうちコンサルト

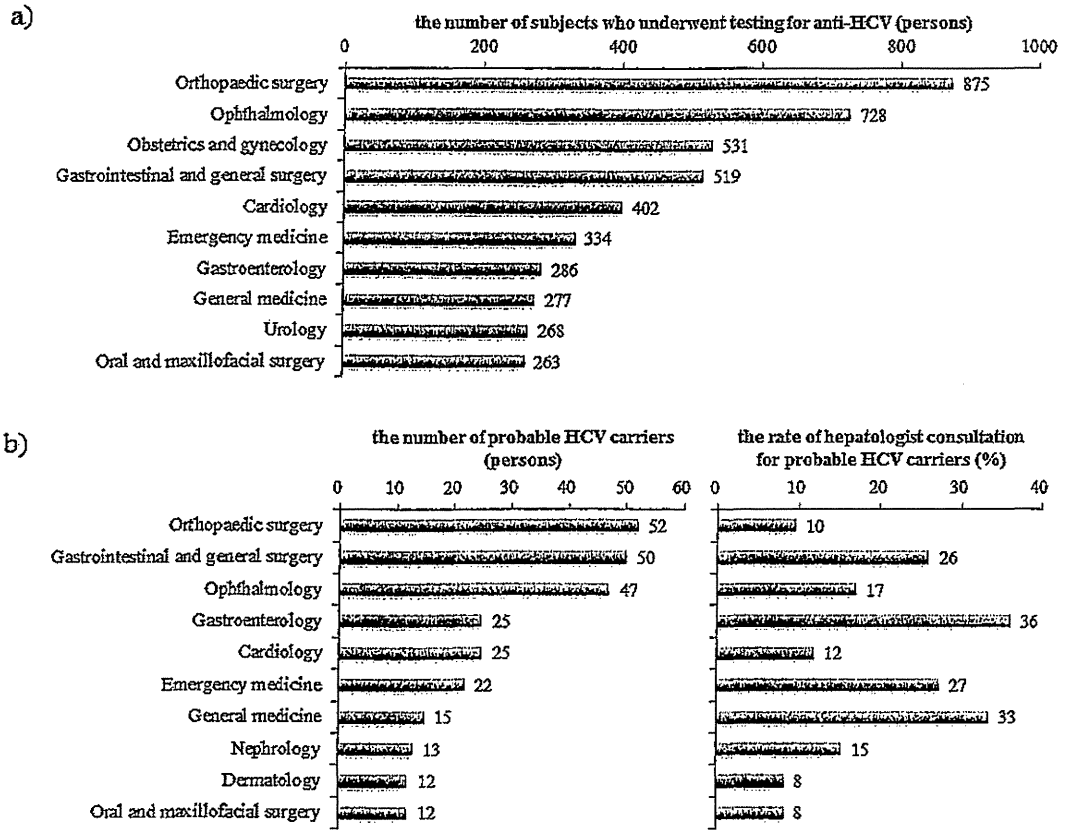


**Fig. 3** a) The number of subjects who underwent anti-HCV screening test in the top 10 non-hepatology departments. b) The number of probable HCV carriers in the top 10 non-hepatology departments, and the rate of hepatologist consultation.

**Table 5** Characteristics of probable HCV carriers according to hepatologist consultation (n = 369)

| | with consultation n = 67 | without consultation n = 302 | *P*-value |
|---|---|---|---|
| Age (years) | 69 (33-85) | 71 (0-93) | 0.113 |
| Males/females | 38/29 | 156/146 | 0.500 |
| Department: Internal Medicine/others | 25/84 | 42/218 | 0.139 |
| Platelet ($\times 10^4/\mu L$) | 13.9 (5.5-38.8) | 16.7 (2.7-62.6) | 0.014 |
| AST (IU/L) | 38 (13-116) | 29 (10-822) | 0.017 |
| ALT (IU/L) | 31 (5-162) | 24 (4-230) | 0.024 |
| AST/ALT ratio | 1.21 (0.41-3.00) | 1.31 (0.34-7.76) | 0.266 |
| γ-GTP (IU/L) | 30 (9-190)† | 23 (7-564)‡ | 0.042 |
| Anti-HCV (S/CO) | 14.2 (5.6-18.8) | 13.4 (4.0-18.5) | <0.001 |
| with/without hepatitis | 46/21 | 198/104 | 0.671 |

Values are median (range) or number of patients. †n = 59, ‡n = 257.

に寄与する因子の多変量解析では，Anti-HCV（OR：1.200，95%CI：1.019–1.413，$p = 0.029$）が唯一関与していた．

非介入群の診療内容調査では，150例（50%）はHCVについて全く記載がなく，63例（21%）は既往歴に記載があるのみでプロブレムリストには挙げられておらず，79例（26%）はプロブレムリストに挙げられているものの診療方針が記載されていなかった（Table 8）．

**Table 6** Univariate and multivariate analysis of factors associated with hepatologist consultation for probable HCV carriers (n＝369)

| Factors | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value |
| Age | 0.989 (0.969-1.009) | 0.265 | | |
| Sex: male | 1.226 (0.719-2.091) | 0.453 | | |
| Department: except internal medicine | 0.647 (0.371-1.128) | 0.125 | 0.576 (0.320-1.034) | 0.065 |
| Platelet ($\times 10^4/\mu L$) | 0.966 (0.927-1.006) | 0.094 | 0.986 (0.948-1.026) | 0.487 |
| AST (IU/L) | 1.002 (0.997-1.007) | 0.382 | | |
| ALT (IU/L) | 1.017 (1.007-1.027) | ＜0.001 | 1.014 (1.004-1.024) | 0.007 |
| γ-GTP (IU/L)† | 1.001 (0.997-1.005) | 0.744 | | |
| Anti-HCV (S/CO) | 1.219 (1.096-1.355) | ＜0.001 | 1.196 (1.073-1.334) | 0.001 |

†n＝316.

**Table 7** Univariate and multivariate analysis of factors associated with hepatologist consultation for probable chronic hepatitis C individuals (n＝244)

| Factors | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value |
| Age | 0.990 (0.965-1.015) | 0.432 | | |
| Sex: male | 1.206 (0.622-2.338) | 0.580 | | |
| Department: except Internal Medicine | 0.724 (0.370-1.418) | 0.346 | | |
| Platelet ($\times 10^4/\mu L$) | 0.902 (0.839-0.969) | 0.005 | 0.897 (0.831-0.968) | 0.005 |
| AST (IU/L) | 1.002 (0.997-1.007) | 0.357 | | |
| ALT (IU/L) | 1.022 (1.010-1.034) | ＜0.001 | 1.020 (1.008-1.033) | 0.001 |
| γ-GTP (IU/L)† | 1.000 (0.996-1.005) | 0.877 | | |
| Anti-HCV (S/CO) | 1.290 (1.108-1.503) | 0.001 | 1.283 (1.085-1.518) | 0.004 |

†n＝211.

**Table 8** Situation of medical practice for probable HCV carriers without hepatologist consultation (n＝302)

| Descriptions regarding HCV on electronic medical record | n (%) |
|---|---|
| nothing | 150 (50) |
| written in the past history, but not listed on the problem lists | 63 (21) |
| listed on the problem lists, but not mentioned in the plan for medical practice | 79 (26) |
| listed on the problem lists and mentioned in the plan for medical practice | 10 (3) |

## 考　察

今回我々が行った院内調査では，HBsAg陽性率1.9%，推定HCVキャリア率5.6%であり，平成18年度の全国調査における結果(HBsAg陽性率：全国1.0%，佐賀県1.8%；推定HCVキャリア率：全国0.8%，佐賀県2.7%)[6]に比して，高率であった．特に，C型肝炎が高率だが，これは大学病院受診者に高齢者が多いことに起因していると推測される．実際，推定HCVキャリアの年齢中央値は70歳であるが，今回の結果は佐賀県の最近10年間(2001～2010年）の70歳代におけるHCVキャリア率(5.2%)とほぼ一致する(未発表データ)．このように，院内で多くの肝炎ウイルスキャリアが判明しているに

もかかわらず，HBVキャリアの79%および推定HCVキャリアの82%で肝臓内科との連携がなく，そのうち，前者の89%および後者の97%において肝炎に関する診療方針も記載されていない状況であった．但し，カルテに記載のない症例においても，患者へ結果説明はなされている症例が存在すると推測され，今回の後ろ向き研究では結果の開示率は不明である．今後，スクリーニングで判明した陽性者が適切な治療まで結びついているかの追跡調査が必要と考えられる．

科別の測定数では，両検査とも整形外科，眼科，産婦人科，一般・消化器外科，循環器内科の順で多く，これらはほとんどが術前スクリーニング検査と推測される．HBVキャリアの肝臓内科へのコンサルトに関与する因子として，年齢と非内科系診療科が負の因子として抽出され，推定B型慢性肝炎例では，非内科系診療科のみが負の傾向を認めた．HBVキャリアの多い10診療科をみても，非内科系診療科はコンサルト率が低く，特に整形外科や眼科で低率であり，これらの診療科への働きかけが重要と考えられる．B型肝炎は複雑な病態を示すため[12]，HBsAg陽性（HBVキャリア）であれば肝臓専門医へのコンサルトが必須である．また，B型肝炎は家族内感染が多く[12]，さらに多くのHBVキャリアの掘り起しに繋がることも重要な点である．推定HCVキャリア全体ではコンサルトに関与する因子としてALT値とHCV抗体価が独立した因子であり，非内科系診療科も負の傾向を認めた．推定C型慢性肝炎例では，肝炎の活動性や線維化が軽度な症例ではコンサルトがされにくい状況が明らかとなった．C型肝炎は，ほとんどの症例で徐々に線維化の進展を来し，それに伴い肝発癌率が上昇していくため[12]，今回C型慢性肝炎と推定された症例では，肝臓専門医の介入は必須である．また，ALT持続正常例においても緩徐ではあるが肝線維化は進行するため[13]，慢性肝炎の可能性が低いとみなされたHCVキャリア症例の中にも，抗ウイルス治療を要する症例が存在する可能性は十分にあり，全科へ積極的な肝臓内科へのコンサルトを啓発していくことが重要である．

Anti-HCV感染率は腎臓内科で最も高いが，これは透析患者の高い罹患率やHCVの肝外病変としての腎疾患を反映しているものと推測される．透析患者においてもHCV感染が生命予後を悪化させることが明らかにされており，抗ウイルス療法を行うことが推奨されている[14]．また，透析患者では血清トランスアミナーゼ値が低値であるため[14]，治療を行うべき症例が見落とされている可能性もあり，肝臓専門医の判断が必要である．一般・消化器外科ではHBsAg，Anti-HCVともに陽性者が多いが，肝癌に対する手術が行われている影響と考えられ，これらの患者は肝疾患専門のかかりつけ医を受診している可能性が高い．一方で，anti-HCV陽性者の多い眼科や整形外科では肝疾患として医療機関を受診している可能性が低いことが危惧される．眼科における感染症スクリーニングの報告は散見されるが，HBsAg陽性率0.5～1.4%，Anti-HCV陽性率4.3～5.8%と，我々の結果と同様にHCVにおいて高率である[15)~18)]．しかし，いずれの報告も医療者側の感染予防対策に論点が限られており，肝炎ウイルスキャリアの肝炎診療についての言及は皆無であった．特に整形外科など非内科系診療科では同様の傾向にあるものと推測され，肝炎ウイルス検査を行うことの多い診療科に対して，検査陽性時の対応を明確に示しておくことが重要であると考えられる．

これまで，肝炎対策の問題点として，検診における肝炎ウイルス検査受検率や要精密者の医療機関受診率の低さ，さらには一般医療機関での肝疾患診療の不備などが指摘されてきた[8]．ところが，大学病院のような高次医療機関においても，多くの肝炎ウイルスキャリアが肝疾患診療に結びついていないという事実が，今回明らかとなった．これまで当院で非肝臓内科において専門的な情報提供や精査の機会を逸した肝炎ウイルス陽性患者については，倫理委員会の承認の元，可能な限り遡及調査とフォローアップを行うべく，まず平成20年度以降の現在の電子カルテからの抽出作業に着手している．さらに，厚生労働省が平成23年度の施策として，肝炎治療促進の環境整備のために「地域肝炎治療コーディネーター養成事業」を挙げ，佐賀県においても，135名のコーディネーターを養成し，当院でも活躍しているところであり，現在の診療データでの評価が可能な症例や通院を継続している症例については，可能な限り主診療科と主治医の協力の元，肝炎コーディネーターによる肝疾患の受療の調査および情報提供を行うことを計画している．しかし，大学病院の特性から追跡困難な症例もあり，その場合は，可能な限り，紹介先の医療機関への情報提供を行うべきであろうと考える．またそれらの症例の中では，測定時と比べて肝疾患の進展を来している症例も少なからずは存在することが想定され，それらの症例へは個々に最善のアプローチと対策が強く望まれる．プロスペクティブな体制としては，現在，外来診療委員会から各科への肝

炎ウイルス陽性者へのコンサルテーション推進の周知がなされ，肝炎コーディネーターへの介入依頼が見られる．さらに，次期電子カルテシステムではウイルス陽性者が発生した際に，電子カルテ上で自動的に主治医に通知し，肝臓専門医へのコンサルテーションを促すシステムと主治医の依頼によって肝炎コーディネーターが対象者に対する個別指導を行うための指導ツールの開発，運用を検討している．肝炎ウイルスキャリア率が高く，かつ肝臓専門医を擁する医療機関において，肝炎ウイルスキャリアに対する適切な肝炎診療を行うための院内連携システムを構築することは，肝炎対策において非常に重要な位置を占めるものと考えられる．

## 結　語

大学病院の非肝臓内科において，多くのHBsAgおよびAnti-HCVが測定されていたが，その陽性率が非常に高いにもかかわらず，介入を要すると推測される肝炎ウイルスキャリア症例の約8割が肝臓内科に紹介されていないという事実が明らかとなった．非肝臓内科におけるHBsAgおよびAnti-HCV陽性者を適切な肝疾患診療に結びつける院内連携システムの構築が，今後，早急に必要である．

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本論文内容に関連する著者の利益相反：なし

# Current management practices for HBs antigen or anti-HCV antibody positive individuals in non-hepatology departments at a university hospital

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Little is known about the current medical management practices relating to hepatitis virus carriers in non-hepatology departments. The aim of this study was to clarify the existing management of viral hepatitis in non-hepatology departments at a university hospital. Subjects who underwent screening tests for HBsAg (n＝6,648) and anti-HCV (n＝6,612) at 27 non-hepatology departments between January 2010 and December 2010 were analyzed. The number of HBsAg-positive (HBV carrier), probable chronic hepatitis B, anti-HCV-positive, probable HCV carrier, and probable chronic hepatitis C were 126 (1.9%), 66 (1.0%), 487 (7.4%), 369 (5.6%), and 244 (3.7%), respectively. In spite of high infection rates, 79% of HBV carriers and 82% of probable HCV carriers were not referred to a hepatologist. In 89% of the former and 97% of the latter, a medical plan for viral hepatitis was not described in the electronic medical record. A system to manage hepatitis virus carriers should be established immediately in medical institutions that have hepatologists.

**Key words:** hepatitis virus　HBs antigen　anti-HCV antibody　screening　management of hepatitis virus carriers

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AUTOIMMUNE, CHOLESTATIC, AND BILIARY DISEASE

# Anticholestatic Effects of Bezafibrate in Patients with Primary Biliary Cirrhosis Treated with Ursodeoxycholic Acid

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**Bezafibrate is a widely used hypolipidemic agent and is known as a ligand of the peroxisome proliferator-activated receptors (PPARs). Recently this agent has come to be recognized as a potential anticholestatic medicine for the treatment of primary biliary cirrhosis (PBC) that does not respond sufficiently to ursodeoxycholic acid (UDCA) monotherapy. The aim of this study was to explore the anticholestatic mechanisms of bezafibrate by analyzing serum lipid biomarkers in PBC patients and by cell-based enzymatic and gene expression assays. Nineteen patients with early-stage PBC and an incomplete biochemical response to UDCA (600 mg/day) monotherapy were treated with the same dose of UDCA plus bezafibrate (400 mg/day) for 3 months. In addition to the significant improvement of serum biliary enzymes, immunoglobulin M (IgM), cholesterol, and triglyceride concentrations in patients treated with bezafibrate, reduction of 7α-hydroxy-4-cholesten-3-one (C4), a marker of bile acid synthesis, and increase of 4β-hydroxycholesterol, a marker of CYP3A4/5 activity, were observed. *In vitro* experiments using human hepatoma cell lines demonstrated that bezafibrate controlled the target genes of PPARα, as well as those of the pregnane X receptor (PXR); down-regulating CYP7A1, CYP27A1, and sinusoidal $Na^+$/taurocholate cotransporting polypeptide (NTCP), and up-regulating CYP3A4, canalicular multidrug resistance protein 3 (MDR3), MDR1, and multidrug resistance-associated protein 2 (MRP2). *Conclusion:* Bezafibrate is a dual PPARs/PXR agonist with potent anticholestatic efficacy in early-stage PBC patients with an incomplete biochemical response to UDCA monotherapy. (HEPATOLOGY 2013;57:1931-1941)**

See Editorial on Page 1691

Primary biliary cirrhosis (PBC) is a chronic liver disease that is presumably caused by autoimmunity. The detection of serum antimitochondrial antibodies (AMA) and increased levels of immunoglobulin M (IgM) are biochemical features of this disease. Histopathologically, it is characterized by portal inflammation and the slow progressive destruction of the portal interlobular bile ducts due to chronic nonsuppurative cholangitis. The loss of bile ducts leads to cholestasis, which leads to further hepatic damage, fibrosis, cirrhosis, and ultimately, liver failure.[1]

Ursodeoxycholic acid (UDCA) is the only Food and Drug Administration (FDA)-approved drug and the first-line medicine for the treatment of PBC.[2] UDCA has been shown to improve serum levels of biliary enzymes and IgM, and may slow the histologic progression to liver cirrhosis.[3-6] The mechanisms of the

*Abbreviations: ABC, ATP-binding cassette transporter; BSEP, bile salt export pump; C4, 7α-hydroxy-4-cholesten-3-one; CA, cholic acid; CAR, constitutive androstane receptor; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; FGF, fibroblast growth factor; FXR, farnesoid X receptor; 4β-HC, 4β-hydroxycholesterol; 24S-HC, 24S-hydroxycholesterol; 27-HC, 27-hydroxycholesterol; HMGCR, HMG-CoA reductase; HNF4α, hepatocyte nuclear factor 4α; LCA, lithocholic acid; LXRα, liver X receptor α; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; NF-κB, nuclear factor-κB; NTCP, $Na^+$/taurocholate cotransporting polypeptide; PBC, primary biliary cirrhosis; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; PGC1α, peroxisome proliferator-activated receptor-γ coactivator-1α; UDCA, ursodeoxycholic acid.*

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anticholestatic and antiinflammatory effects of UDCA have been reported to be due to the activation of the canalicular bile salt export pump (BSEP), canalicular multidrug resistance protein 3 (MDR3; ATP-binding cassette transporter B4 [ABCB4]) and basolateral multidrug resistance-associated protein 4 (MRP4 [ABCC4]).[7] In addition, the replacement of hydrophobic bile acids with hydrophilic UDCA appears to attenuate the damage to hepatocytes and biliary cells.[2] It has been reported that about two-thirds of patients treated with UDCA in the early stage of the disease could have a normal life expectancy without additional therapies.[8] However, the remaining patients are not sufficiently controlled with UDCA monotherapy and additional therapeutic approaches have been necessary.

Immunosuppressive medication is not recommended as the first-line, alternative drug for PBC, but budesonide, a nonhalogenated glucocorticoid with a high first-pass metabolism, and/or mycophenolate mofetil, an inhibitor of the purine biosynthetic pathway which is critical to lymphocytic proliferation and activation, are sometimes used in patients who fail to respond to UDCA.[9,10] However, the effects of these immunosuppressive agents remain controversial.[11,12] The farnesoid X receptor (FXR; NR1H4) agonist, 6-ethyl-chenodeoxycholic acid, has been administered to PBC patients that exhibit incomplete responses to UDCA in a phase II clinical trial. This trial exhibited anticholestatic effects and serum alkaline phosphatase (ALP) levels were reduced, but pruritus occurs at the higher doses.[13]

In 1999, Iwasaki et al.[14] introduced the effectiveness of a hypolipidemic agent, bezafibrate, on the reduction of serum ALP and IgM levels in precirrhosis PBC patients, and recently, combination therapy with UDCA and bezafibrate is being recognized as a beneficial treatment for PBC that is refractory to UDCA monotherapy.[15,16] Although the mechanisms of anticholestatic action by bezafibrate have not been elucidated completely, it is believed that the induction of MDR3 through activation of the peroxisome proliferator-activated receptor α (PPARα; NR1C1)[17] is the main mechanism, because fibrate class agents are ligands of the PPARs.[18] However, because MDR3 is activated by both the addition of bezafibrate as well as by UDCA monotherapy,[7] the roles of bezafibrate in the combination therapy remain unknown.

The current study was undertaken to explore the mechanisms of the remission of cholestasis by bezafibrate in PBC patients who failed to respond to UDCA monotherapy. Our *in vivo* and *in vitro* studies demonstrated that bezafibrate was a dual PPARs/pregnane X receptor (PXR; NR1I2) agonist with potent anticholestatic efficacy.

## Patients and Methods

***Patients.*** Thirty-one Japanese patients with asymptomatic and untreated PBC (4 males and 27 females; ages 37-81 years) were enrolled in the study. The diagnosis of PBC was established by laboratory and histological findings, and all patients were classified as early-stage PBC (Scheuer's classification I or II). Informed consent was obtained from all subjects and the study protocol was approved by the Ethics Committee of Tokyo Medical University Ibaraki Medical Center.

***Study Design.*** All patients (n = 31) were treated with UDCA (600 mg/day; 10-13 mg/kg/day) alone for at least 3 months (maximum 6 months) until serum ALP and gamma glutamyl transpeptidase (GGT) became stable (Supporting Figure). Then bezafibrate (400 mg/day) was administered with UDCA (600 mg/day) to patients (n = 19; 1 male and 18 females) who exhibited an incomplete biochemical response to UDCA monotherapy (defined as ALP or GGT level of above the upper limit of normal) and treated for 3 months. Before and after UDCA monotherapy and after the addition of bezafibrate, blood samples were collected in the morning before breakfast after an overnight fasting, and serum was stored at −20°C until analyzed. Control sera from 49 healthy Japanese volunteers (11 males and 38 females; ages 22-79 years) were obtained from another study group (courtesy of Prof. T. Teramoto, Teikyo University School of Medicine, Tokyo, Japan) and were stored as mentioned above.

***Determination of Serum Markers for Cholesterol and Bile Acid Metabolism.*** Serum sterol concentrations were determined by liquid chromatography, tandem mass spectrometry (LC-MS/MS) as described.[19] Serum fibroblast growth factor 19 (FGF19) levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantikine Human

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FGF-19 Immunoassay, R&D Systems, Minneapolis, MN). Serum bile acid profiles were determined by LC-MS/MS according to the method of Ando et al.[20]

***Cell Culture.*** The human hepatoma cell line, HepaRG, was obtained from Biopredic International (Rennes, France). On day 0 a 24-well plate was seeded with $4.8 \times 10^5$ differentiated HepaRG cells/well using HepaRG Thawing and Seeding Medium 670. On day 3 the medium was replaced with 500 μL/well of HepaRG Induction Medium 640 containing bezafibrate, rifampicin, carbamazepine, or GW4064 dissolved in 1% acetonitrile. Cells were incubated for 48 hours at 37°C in a humidified incubator containing 5% $CO_2$ and 95% air.

***Assays of CYP3A4 Activity and PXR Activation.*** CYP3A4 activities were measured by cell-based P450-Glo CYP3A4 Assay Kit (Luciferin-IPA) purchased from Promega (Madison, WI). The activation of PXR was determined by a Human PXR Activation Assay System (Puracyp, Carlsbad, CA) utilizing DPX2 hepatoma cells harboring the human PXR and luciferase-linked CYP3A4 promoters.

***RNA Measurements.*** Total RNA was extracted from the HepaRG cells using an RNeasy Plus Mini Kit (Qiagen, Tokyo, Japan). Reverse transcription and real-time quantitative polymerase chain reaction (PCR) were performed as described.[21] The sequences of some primer pairs have been described in the same report.[21] The other primer sequences used in this study are listed in the Supporting Table.

***Statistics.*** Data are reported as the mean ± SEM for human data and as the mean ± SD for cell data. The statistical significance of differences between the results in the different groups was evaluated by non-parametric Mann-Whitney test for human data (Tables 1, 2) and Student's two-tailed $t$ test for cell data (Figs. 4, 5). On the other hand, the data obtained before and after treatment were compared by Wilcoxon signed-ranks test (Figs. 1-3). In all statistical tests significance was accepted at the level of $P < 0.05$.

## Results

The characteristics of the PBC patients enrolled in the present study are shown in Table 1. In patients before UDCA treatment (n = 31) and those who responded to UDCA insufficiently and before additional bezafibrate treatment (n = 19), serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), GGT, ALP, and IgM levels were significantly elevated compared with healthy controls. Serum low-density lipoprotein (LDL) cholesterol and triglyceride concentrations were increased and HDL cholesterol concentration was decreased significantly in the patients before UDCA treatment compared with controls. In the patients before additional bezafibrate treatment a similar tendency was observed, but the differences were not statistically significant.

Baseline biomarker levels for lipid metabolism in the three groups are compared in Table 2. In this study cholesterol metabolism in PBC patients was assayed by measuring serum sterol biomarkers. Because most non-cholesterol sterols are transported in serum with cholesterol, the expression of each sterol level relative to the total cholesterol concentration tends to be more reliable compared with the absolute concentration, especially when dyslipidemia is present.[22] Serum concentrations

**Table 1. Characteristics of Patients with PBC Enrolled in the Present Study**

| Laboratory Data | Control (n=49) | Before UDCA Treatment (n=31) | Before BF Treatment (n=19) |
|---|---|---|---|
| Age (yrs) | 57.8±1.6 [22-79] | 60.3±1.8 [37-81] | 58.8±1.6 [45-73] |
| Gender (Male/Female) | 11/38 | 4/27 | 1/18 |
| AST (IU/L) | 21±1 [11-34] | 64±18‡ [19-120] | 45±5‡ [20-101] |
| ALT (IU/L) | 17±1 [7-30] | 82±34‡ [12-138] | 51±9‡ [18-152] |
| GGT (IU/L) | 25±2 [7-58] | 196±27‡ [30-757] | 178±59‡ [47-445] |
| ALP (IU/L) | 230±9 [126-336] | 517±43‡ [229-1163] | 597±51‡ [266-952] |
| Total bilirubin (mg/dL) | 0.7±0.1 [0.3-1.2] | 0.7±0.2 [0.3-1.3] | 0.6±0.1 [0.3-1.1] |
| IgM (mg/dL) | 97±12 [56-161] | 288±27‡ [90-637] | 306±60† [130-466] |
| Total cholesterol (mg/dL) | 199±4 [130-257] | 213±9 [120-356] | 228±18 [118-343] |
| LDL cholesterol (mg/dL) | 115±4 [46-194] | 138±7* [91-254] | 149±18 [54-228] |
| HDL cholesterol (mg/dL) | 65±2 [33-111] | 53±4* [13-95] | 55±5 [13-89] |
| Triglycerides (mg/dL) | 91±6 [33-214] | 107±7* [47-199] | 113±11 [40-243] |

Data are expressed as mean ± SEM [range].

Before UDCA treatment, all PBC patients before treatment with UDCA; Before BF treatment, PBC patients who exhibited an incomplete biochemical response to the UDCA monotherapy (600 mg/day) and before additional treatment with bezafibrate.

*$P < 0.05$, significantly different from control.

†$P < 0.005$, significantly different from control.

‡$P < 0.0001$, significantly different from control.

**Table 2. Baseline Biomarker Levels for Cholesterol Metabolism in Enrolled Patients with PBC**

| Serum Biomarkers | Control (n=49) | Before UDCA Treatment (n=31) | Before BF Treatment (n=19) |
|---|---|---|---|
| Bile acid metabolism | | | |
| C4 (ng/mg CHOL) | 15.7±2.9 [2.3-118] | 12.1±1.8 [0.8-49] | 11.8±2.1 [1.5-38] |
| FGF19 (pg/ml) | 336±51 [50-1662] | 309±49 [74-1543] | 353±57 [114-930] |
| Cholesterol metabolism | | | |
| Lathosterol (μg/mg CHOL) | 2.8±0.3 [0.9-11.7] | 2.2±0.2 [0.7-5.8] | 2.2±0.3 [0.8-6.1] |
| Sitosterol (μg/mg CHOL) | 1.6±0.1 [0.4-3.8] | 2.0±0.2* [0.8-3.9] | 2.4±0.2† [1.1-4.3] |
| Campesterol (μg/mg CHOL) | 1.8±0.1 [0.4-5.1] | 2.0±0.1 [0.7-3.7] | 1.9±0.2 [0.7-3.3] |
| Oxysterol metabolism | | | |
| 4β-HC (ng/mg CHOL) | 29±3 [11-135] | 44±4‡ [24-140] | 51±5‡ [20-92] |
| 24S-HC (ng/mg CHOL) | 31±2 [17-74] | 34±2 [22-69] | 41±2†[20-64] |
| 27-HC (ng/mg CHOL) | 77±3 [35-140] | 75±4 [48-124] | 75±4 [39-102] |

Data are expressed as mean ± SEM [range].

Before UDCA treatment, all PBC patients before treatment with UDCA; Before BF treatment, PBC patients who exhibited an incomplete biochemical response to the UDCA monotherapy (600 mg/day) and before additional treatment with bezafibrate; C4, 7α-hydroxy-4-cholesten-3-one; CHOL, cholesterol; FGF19, fibroblast growth factor 19; 4β-HC, 4β-hydroxycholesterol; 24S-HC, 24S-hydroxycholesterol; 27-HC, 27-hydroxycholesterol.

*$P < 0.05$, significantly different from control.

†$P < 0.005$, significantly different from control.

‡$P < 0.0001$, significantly different from control.

of sitosterol, 4β-hydroxycholesterol (4β-HC), and 24S-hydroxycholesterol (24S-HC) expressed relative to total cholesterol were significantly elevated in both patient groups compared with controls. However, other sterols, 7α-hydroxy-4-cholesten-3-one (C4), lathosterol, campesterol, and 27-hydroxycholesterol (27-HC), and FGF19 concentrations did not differ significantly among the three groups.

***Effects of UDCA and Bezafibrate on Serum Liver Enzymes and Lipids.*** As shown in Fig. 1A, serum AST, ALT, GGT, ALP, and IgM levels were all reduced significantly by treatment with UDCA. In patients

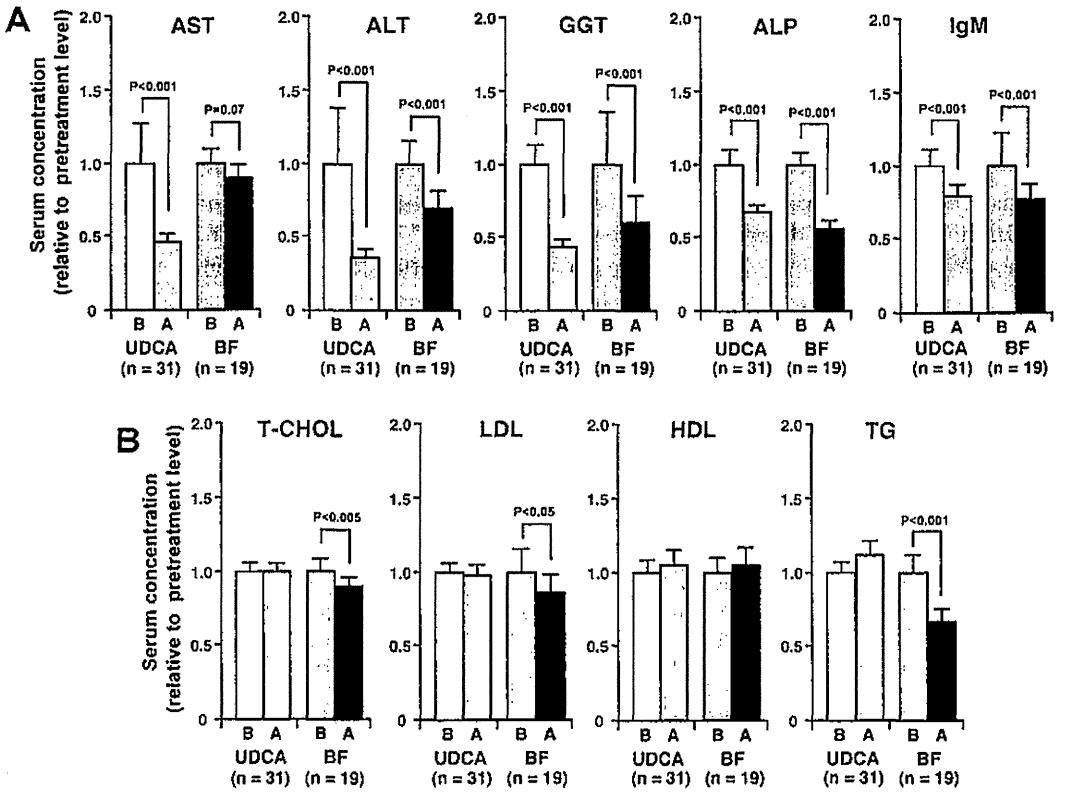


Fig. 1. Effects of UDCA and additional bezafibrate treatment on serum liver enzymes (A) and lipids (B). B, before treatment; A, after treatment; BF, bezafibrate; T-CHOL, total cholesterol; LDL, LDL cholesterol; HDL, HDL cholesterol; TG, triglyceride. The mean concentrations before treatment were set to 1.0, and the absolute concentrations before treatment are shown in Table 1. Data are expressed as the mean ± SEM.

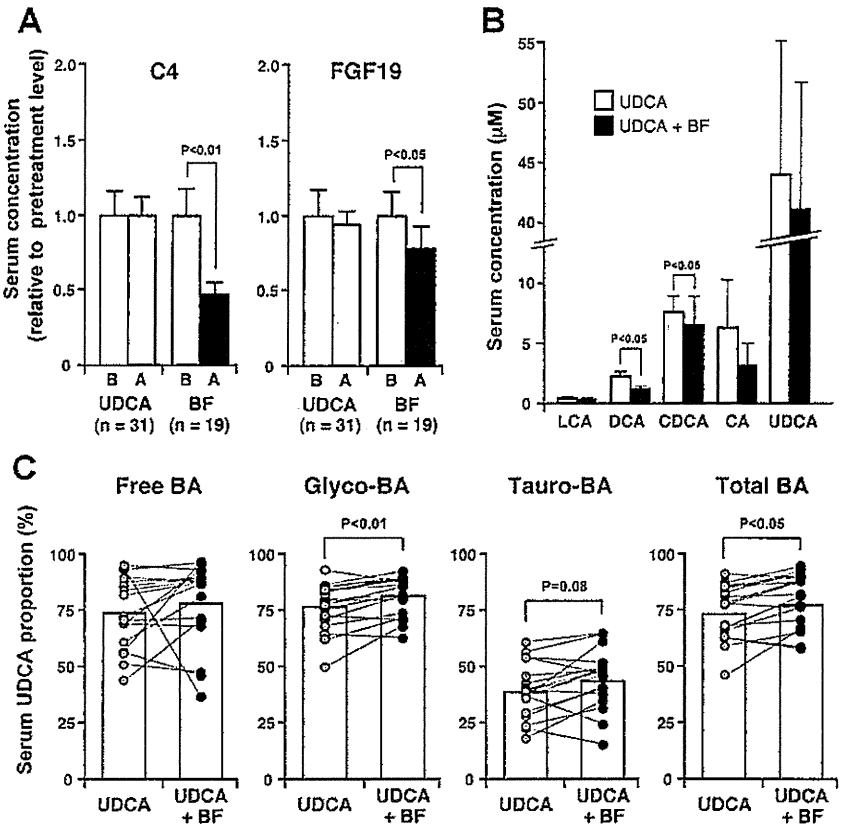


Fig. 2. Effects of UDCA and additional bezafibrate treatment on bile acid metabolism. (A) C4, 7α-hydroxy-4-cholesten-3-one; FGF19, fibroblast growth factor 19; B, before treatment; A, after treatment; BF, bezafibrate. Mean concentrations before treatment (ng/mg cholesterol for C4 and pg/ml for FGF19) were set to 1.0, and the absolute concentrations before treatment are shown in Table 2. Data are expressed as the mean ± SEM. (B) Serum concentrations of bile acids in UDCA-treated patients before and after addition of bezafibrate (n = 17). (C) Serum proportions of UDCA in UDCA-treated patients before and after addition of bezafibrate (n = 17). The mean value for each group is indicated by the columns. Free BA, unconjugated bile acids; Glyco-BA, glycine-conjugated bile acids; Tauro-BA, taurine-conjugated bile acids.[1]

who responded incompletely to UDCA monotherapy, the combination of bezafibrate and UDCA further reduced serum levels of ALT, GGT, ALP, and IgM. The changes in serum lipid concentrations by UDCA and bezafibrate treatment are presented in Fig. 1B. UDCA monotherapy did not change the serum lipid levels significantly. However, the addition of bezafibrate significantly decreased serum concentrations of total cholesterol, LDL cholesterol, and triglyceride in those patients whose cholestasis was not sufficiently improved by UDCA alone.

***Effects of UDCA and Bezafibrate on Bile Acid Metabolism.*** C4 and FGF19 are markers of bile acid production[23] and transintestinal flux,[24] respectively. As shown in Fig. 2A, UDCA did not change C4 or FGF19 concentrations, but bezafibrate significantly reduced both C4 and FGF19 levels. In Fig. 2B,C, serum bile acid concentrations and UDCA proportion in UDCA-treated patients before and after addition of bezafibrate are shown. The addition of bezafibrate significantly reduced the serum chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA) concentrations. The serum cholic acid (CA) and lithocholic acid (LCA) concentrations also tended to be reduced by bezafibrate, but the differences were not statistically significant. The serum proportion of UDCA was significantly increased by the addition of bezafibrate compared with UDCA monotherapy, presumably due to its inhibitory effect on *de novo* bile acid biosynthesis. The proportion of UDCA in serum is usually higher than that in bile in patients treated with UDCA, but it appears to reflect the biliary proportion of UDCA to some extent.[25]

***Effects of UDCA and Bezafibrate on Sterol Metabolism.*** Cholesterol biosynthesis and intestinal absorption were studied by measuring serum concentrations of lathosterol and plant sterols (sitosterol and campesterol), respectively. As shown in Fig. 3A, UDCA treatment did not affect cholesterol biosynthesis but significantly increased cholesterol absorption. In contrast, bezafibrate significantly inhibited cholesterol biosynthesis but did not change cholesterol absorption.

Serum concentrations of major oxysterols that are potential ligands of liver X receptor α (LXRα, NR1H3) were compared between UDCA and bezafibrate treatments (Fig. 3B). UDCA treatment did not affect serum 4β-HC or 24S-HC concentrations but increased the 27-HC concentration significantly. Treatment with bezafibrate clearly increased serum 4β-HC

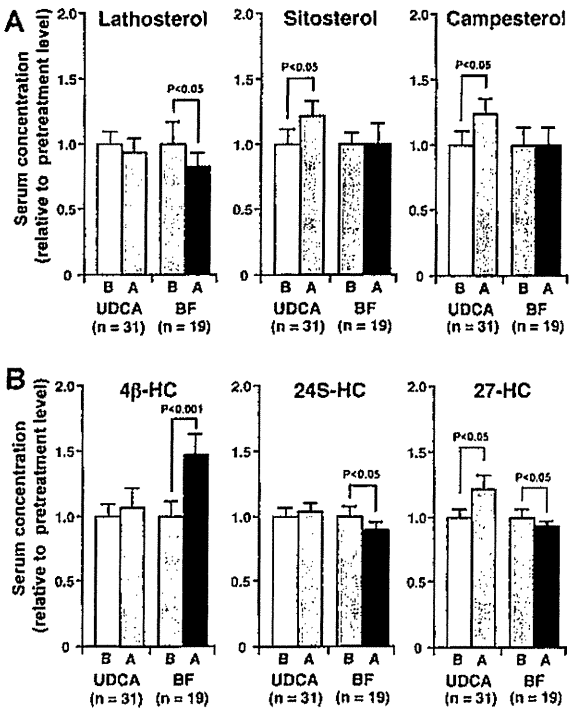


Fig. 3. Effect of UDCA and additional bezafibrate treatment on cholesterol (A) and oxysterol (B) metabolism. B, before treatment; A, after treatment; BF, bezafibrate; 4β-HC, 4β-hydroxycholesterol; 24S-HC, 24S-hydroxycholesterol; 27-HC, 27-hydroxycholesterol. Mean concentrations before treatment (μg/mg cholesterol or ng/mg cholesterol) were set to 1.0, and the absolute concentrations before treatment are shown in Table 2. Data are expressed as the mean ± SEM.

levels, whereas it significantly reduced the 24S-HC and 27-HC levels.

***Effects of Bezafibrate on CYP3A4.*** Differentiated HepaRG cells exhibit a gene expression pattern similar to primary human hepatocytes and human liver tissues and maintain significant levels of hepatic cell functions, including CYP and transporter activities.[26] Rifampicin and carbamazepine are classical inducers of CYP3A4 by way of the activation of PXR,[27] whereas GW4064 is one of the most potent agonists of FXR.[28] As shown in Fig. 4A, bezafibrate, as well as rifampicin and carbamazepine, induced both CYP3A4 mRNA expression and activity in a dose-dependent manner.

***Effects of Bezafibrate on PXR Activation.*** The DPX2 cell-based luciferase reporter gene assay demonstrated that in comparison with rifampicin, bezafibrate was a weak but significant activator of human PXR as well as carbamazepine (Fig. 4B). It is noteworthy that GW4064 activated human PXR at concentrations higher than 3 μM.

***Effects of Bezafibrate on Gene Expression of Nuclear Receptors, Transporters, and Enzymes.*** Among the nuclear receptors and related coactivators (Fig. 5A), PXR expression was induced by bezafibrate to a greater degree than that by rifampicin, which suggests that PXR is a target gene of PPARs, as reported.[29] In contrast, the small heterodimer partner (SHP; NR0B2), a

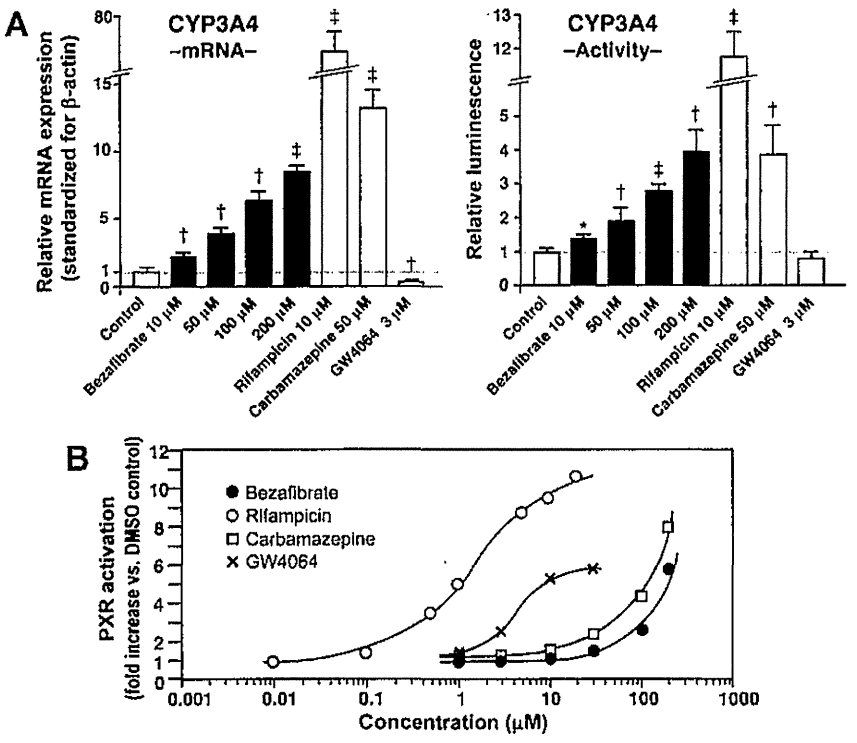


Fig. 4. Effects of bezafibrate, rifampicin, carbamazepine, and GW4064 on the activation of CYP3A4 and human PXR. (A) HepaRG cells were treated with each compound for 48 hours in triplicate. mRNA expression levels were standardized to those of β-actin. The mean expression and activity levels in control cells were set to 1.0. Data are expressed as the mean ± SD. Effects of bezafibrate are shown as solid bars. $^*P < 0.05$, †$P < 0.005$, ‡$P < 0.001$, significant difference from controls. (B) DPX2 cells were treated with each compound for 24 hours in triplicate. Activation of human PXR was determined by a cell-based luciferase reporter gene assay. The average relative luminescent units (RLU) obtained with the dimethyl sulfoxide (DMSO) solvent control was set to 1.0.

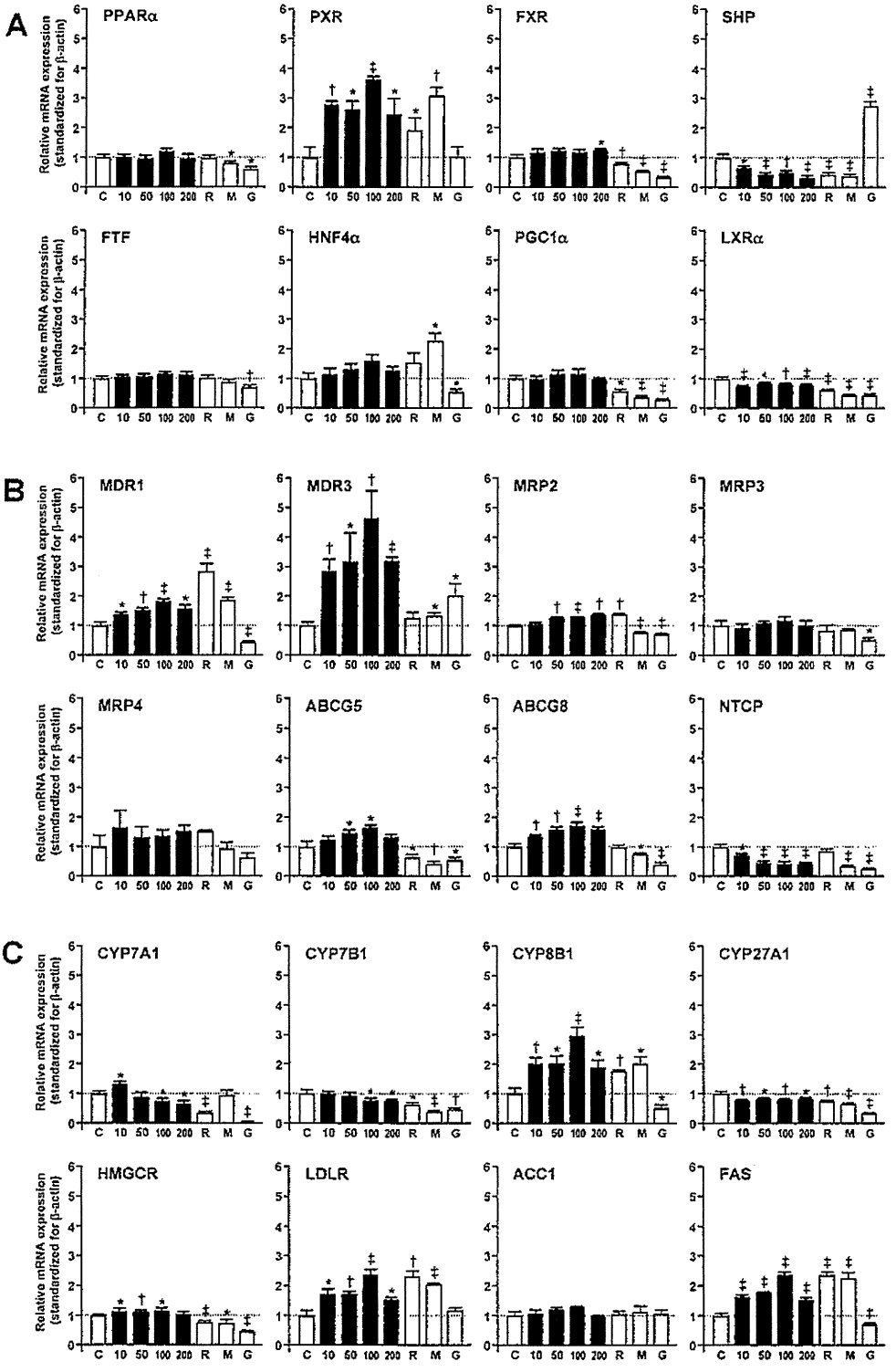


Fig. 5. Effects of bezafibrate, rifampicin, carbamazepine, and GW4064 on mRNA expression levels of nuclear receptors and a related coactivator (A), transporters (B), and enzymes and LDL receptor (C) in HepaRG cells. The cells were treated with each compound for 48 hours, in triplicate. mRNA expression levels were standardized to those of $\beta$-actin. The mean expression and activity levels in control cells were set to 1.0. Data are expressed as the mean ± SD. The effects of bezafibrate are shown as the solid bars. C, control; 10, bezafibrate 10 $\mu$M; 50, bezafibrate 50 $\mu$M; 100, bezafibrate 100 $\mu$M; 200, bezafibrate 200 $\mu$M; R, rifampicin 10 $\mu$M; M, carbamazepine 50 $\mu$M; G, GW4064 3 $\mu$M. PPAR$\alpha$, peroxisome proliferator-activated receptor $\alpha$; PXR, pregnane X receptor; FXR, farnesoid X receptor; SHP, small heterodimer partner; FTF, $\alpha$-fetoprotein transcription factor; HNF4$\alpha$, hepatocyte nuclear factor 4$\alpha$; PGC1$\alpha$, peroxisome proliferator-activated receptor $\gamma$ coactivator 1$\alpha$; LXR$\alpha$, liver X receptor $\alpha$; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; ABC, ATP-binding cassette transporter; NTCP, $Na^+$/taurocholate-cotransporting polypeptide; CYP7A1, cholesterol 7$\alpha$-hydroxylase; CYP7B1, oxysterol 7$\alpha$-hydroxylase; CYP8B1, 7$\alpha$-hydroxy-4-cholesten-3-one 12$\alpha$-hydroxylase; CYP27A1, sterol 27-hydroxylase; HMGCR, HMG-CoA reductase; LDLR, LDL receptor; ACC1, acetyl-CoA carboxylase 1; FAS, fatty acid synthase. *$P < 0.05$, †$P < 0.005$, ‡$P < 0.001$, significant difference from control.

target of FXR, and LXRα were down-regulated by bezafibrate, as well as rifampicin and carbamazepine. FXR and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α) expressions were significantly down-regulated by rifampicin and carbamazepine but not by bezafibrate.

The MDR1 (ABCB1) and MRP2 (ABCC2) transporters (Fig. 5B) were up-regulated by bezafibrate, similar to rifampicin, whereas MDR3, ABCG5, and ABCG8 were up-regulated by bezafibrate but not by rifampicin. In addition, $Na^+$/taurocholate cotransporting polypeptide (NTCP) was down-regulated by bezafibrate but did not change significantly by rifampicin. It is notable that significant messenger RNA (mRNA) expression of BSEP was observed in HepaRG cells treated with GW4064, whereas only a trace amount of BSEP expression was detected in control cells and those treated with other compounds.

Enzymes involved in cholesterol, bile acid, and fatty acid syntheses and LDL receptor expression are summarized in Fig. 5C. CYP7A1, CYP7B1, and CYP27A1 were down-regulated and CYP8B1, fatty acid synthase (FAS), and LDL receptor (LDLR) were up-regulated by bezafibrate, which was the same as the effects of rifampicin. HMG-CoA reductase (HMGCR), the rate-limiting enzyme in the cholesterol biosynthetic pathway, was down-regulated by rifampicin but was slightly up-regulated by bezafibrate.

## Discussion

Our results clearly showed that the combination therapy of bezafibrate and UDCA significantly improved cholestasis in early-stage PBC patients who were refractory to UDCA monotherapy. The mean levels of ALP and GGT during UDCA monotherapy were further reduced from 597 ± 51 to 324 ± 27 IU/L and 178 ± 59 to 99 ± 41 IU/L, respectively, by the additional administration of bezafibrate (Fig. 1). It is known that UDCA not only improves cholestasis but also serum IgM concentrations.[4,6] The combination therapy of bezafibrate and UDCA further reduced the IgM concentration from 306 ± 60 (UDCA alone) to 232 ± 41 mg/dL (UDCA + bezafibrate), consistent with the findings reported by Iwasaki et al.[16] Furthermore, our results showed that the combination therapy significantly reduced serum total cholesterol, LDL cholesterol, and triglyceride concentrations compared with UDCA alone.

The mechanisms of the anticholestatic effect of bezafibrate remain unclear. Because MDR3 is a target gene of PPARα[17] and bezafibrate is a ligand of PPARα, β/δ, and γ,[18] stimulation of biliary phospholipid secretion due to the up-regulation of MDR3 has generally been believed to be the main mechanism of the action. In fact, our experiment using HepaRG cells showed significantly elevated expression of MDR3 mRNA following the addition of bezafibrate (Fig. 5B). However, MDR3 is activated by both bezafibrate as well as UDCA.[7] Furthermore, recent reports have demonstrated that the expression of MDR3 was already markedly up-regulated in PBC patients[30] and it was not significantly affected by bezafibrate treatment.[31] Therefore, the anticholestatic effect of bezafibrate may be caused by mechanisms independent of phospholipid secretion.

Other possible anticholestatic mechanisms of bezafibrate by way of PPARα activation include down-regulation of NTCP,[17] CYP7A1,[32,33] and CYP27A1.[33] NTCP transports basolateral (sinusoidal) bile acids into hepatocytes, whereas CYP7A1 and CYP27A1 are key enzymes in the classic and alternative bile acid biosynthetic pathways, respectively. Coordinate down-regulation of these three proteins leads to a decrease in hepatic bile acid concentration and may protect hepatocytes against cytotoxic bile acids. In addition, the reduction of hepatic bile acid levels attenuates the activity of FXR. It is known that deactivation of FXR up-regulates MRP4,[34] one of the important basolateral transporters for the efflux of bile acids from hepatocytes to the sinusoid in cholestasis. The transcription of MRP4 is positively controlled by the constitutive androstane receptor (CAR; NR1I3)[35] and a CAR responsive element is embedded within an FXR responsive element in the human MRP4 promoter. Therefore, activated FXR competes with CAR for binding to this overlapping binding site, which down-regulates MRP4.[36]

The most striking results among our serum biomarker analyses are the elevation of 4β-HC, as well as the reduction of C4 during treatment with bezafibrate. Serum 4β-HC concentration is considered a biomarker of CYP3A4/5 activity,[37] whereas C4 is a marker of CYP7A1 activity or *de novo* bile acid synthesis.[23] Therefore, the changes in 4β-HC and C4 concentrations during bezafibrate treatment suggest that bezafibrate up-regulates CYP3A4/5 and down-regulates CYP7A1. In fact, our experiments using HepaRG cells clearly demonstrated that bezafibrate induced CYP3A4 mRNA expression and activity (Fig. 4A) and inhibited the expression of CYP7A1 mRNA (Fig. 5C) in a dose-dependent manner. Significant up-regulation of CYP3A4 was caused by at least 10 μM of bezafibrate, whereas the serum peak concentration ($C_{max}$) values after oral administration of 400 mg bezafibrate were 9.1-22.7 μM.[38]

Because the expression of CYP3A4 is mainly controlled by PXR,[39] it was strongly suggested that bezafibrate was a ligand of this nuclear receptor, and this hypothesis was proved by the reporter gene assay (Fig. 4B). In addition to PPARα, PXR also regulates hepatic enzyme and transporter activities to exert protective effects against cholestasis. First, the induced CYP3A4 detoxifies xenobiotics and endogenous substances, including the toxic bile acid LCA.[40,41] The C-6α or C-6β position of LCA is hydroxylated by CYP3A4 and nontoxic hyodeoxycholic acid (6α-OH) or murideoxycholic acid (6β-OH) is formed. Second, the activation of PXR up-regulates MDR1[42] and MRP2,[43] which was also observed in our HepaRG cells treated with rifampicin and bezafibrate (Fig. 5B). MDR1 transports various toxic metabolites and xenobiotics, whereas MRP2 transports organic anions from hepatocytes to bile canaliculi.

These results further suggest that the down-regulation of CYP7A1 by bezafibrate is caused not only by the activation of PPARα but also by the activation of PXR. Li and Chiang[44] demonstrated that hepatocyte nuclear factor 4α (HNF4α; NR2A1) interacts with several coactivators including PGC1α, and that the complex activates the transcription of *CYP7A1* in the absence of ligands.[45] Ligands for PXR activate PXR to promote its interaction with HNF4α, which disrupts the interaction between HNF4α and PGC1α and results in suppression of CYP7A1 expression.

Rifampicin is a more potent ligand of human PXR than bezafibrate (Fig. 4), and has also been shown to have anticholestatic effects in PBC patients.[46] However, continuous administration of rifampicin can sometimes result in severe hepatitis.[47] In addition to rifampicin and bezafibrate, budesonide, but not prednisolone, is also an agonist of the human PXR.[48] Therefore, the therapeutic effects of budesonide on PBC patients may be caused at least in part by the anticholestatic effects by way of the activation of PXR.

Hypercholesterolemia and hypertriglyceridemia are often observed in PBC patients. Although it remains controversial whether or not the lipid abnormalities in this disease increase atherosclerotic risk,[49] the administration of bezafibrate significantly reduced the serum concentrations of LDL cholesterol and triglycerides. The mechanism of the cholesterol-lowering effect of bezafibrate has not yet been completely elucidated, and at the very least, it is not likely due to a direct inhibition of HMGCR[50] (Fig. 5C). Because the concentration of serum lathosterol, a marker for *de novo* cholesterol biosynthesis, was decreased significantly during bezafibrate therapy, inhibition of other enzymes involved in the pathway is strongly suggested. Another mechanism of the cholesterol-lowering effect of bezafibrate may be due to the stimulation of cholesterol efflux from hepatocytes to the bile canaliculi by way of the activation of PPARs. Our experiment using HepaRG cells showed significantly up-regulated expression of ABCG5 and ABCG8 mRNA after bezafibrate but not rifampicin treatment (Fig. 5B). A similar effect of bezafibrate on ABCG5 in human liver has been reported previously.[51]

Because of the inhibition of bile acid synthesis and presumably the stimulation of cholesterol excretion into bile, bezafibrate significantly increases biliary cholesterol saturation.[52] Indeed, increased risk of gallstone formation has been reported in hyperlipidemic patients treated with another fibrate, fenofibrate.[53] However, combination therapy of UDCA and bezafibrate appears to attenuate the adverse effect of bezafibrate, because UDCA markedly lowers biliary cholesterol saturation and dissolves cholesterol gallstones.[2] On the other hand, bezafibrate may augment the anticholestatic and antilithogenic actions of UDCA by inhibiting bile acid synthesis and increasing the proportion of UDCA (Fig. 2C).

In addition to anticholestatic effects, activation of PXR[54] and the PPARs[55] has been reported to suppress inflammation through the inhibition of proinflammatory genes, including nuclear factor-κB (NF-κB), tumor necrosis factor-α, and interleukin-1α. In this study, although we did not evaluate the contribution of the anti-inflammatory effects of bezafibrate to the improvement of biochemical markers, bezafibrate is suggested to be an ideal drug with anticholestatic, hypolipidemic, and even antiinflammatory actions on PBC by way of the activation of both PXR and PPARs.

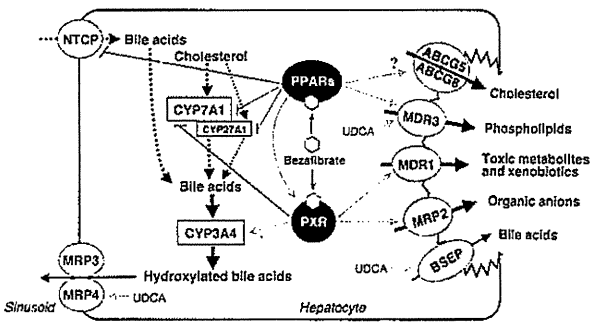


Fig. 6. Regulation of hepatic transporter activities and bile acid metabolism by PPARs, PXR, and UDCA. Bezafibrate is a dual agonist of both PPARs and PXR. The activation of PPARs inhibits CYP7A1, CYP27A1, and NTCP, and up-regulates MDR3, PXR and presumably ABCG5/G8. The activation of PXR inhibits CYP7A1 and stimulates CYP3A4, MDR1, and MRP2. Genes that are down-regulated by PPARs or PXR are indicated by the red lines, whereas those that are up-regulated by PPARs, PXR, or UDCA are indicated by the green arrows.

In summary, bezafibrate exhibited anticholestatic efficacy on PBC patients who showed an incomplete response to UDCA monotherapy. Although UDCA replaces hydrophobic bile acids and activates canalicular BSEP and MDR3 and basolateral MRP4,[7] bezafibrate inhibits hepatic synthesis and uptake of bile acids, enhances bile acid detoxification, and stimulates canalicular MDR3, MDR1 and MRP2 activities as a dual PPARs/PXR agonist (Fig. 6). These data lend support to the idea that combination therapy with UDCA and bezafibrate is an excellent method for the treatment of early-stage PBC patients who exhibit an incomplete biochemical response to UDCA monotherapy.

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# Bile Acid Malabsorption Deactivates Pregnane X Receptor in Patients with Crohn's Disease

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**Background:** Recent studies have suggested that the downregulation of pregnane X receptor (PXR) may contribute to the susceptibility and exacerbation of Crohn's disease (CD). Because bile acid malabsorption is one of the features of CD and bile acids are potential activators of PXR, we explored the relationship between bile acid malabsorption and PXR activities in patients with CD.

**Methods:** Twenty-one patients with CD (4 ileal-resected and 17 nonresected), 10 with ulcerative colitis (UC), and 26 healthy controls were studied. Serum biomarkers for the activity of CYP3A4, a target gene of PXR, and for cholesterol and bile acid metabolism were quantified by liquid chromatography-tandem mass spectrometry or enzyme-linked immunosorbent assay.

**Results:** The concentrations of 4β-hydroxycholesterol (4β-HC), a known marker for CYP3A4 activity, and those of 25-hydroxycholesterol (25-HC), another metabolite by CYP3A4, were significantly reduced in all patients with CD, especially in those with the history of ileal resection. The concentration of 7α-hydroxy-4-cholesten-3-one (C4), a marker for hepatic bile acid biosynthesis, was significantly elevated, whereas the levels of fibroblast growth factor 19 (FGF19), a marker for intestinal bile acid flux, were reduced in patients with CD compared with patients with UC and controls. A significant negative correlation was observed between 4β-HC or 25-HC and C4 concentrations in all patients with CD.

**Conclusions:** The degree of bile acid malabsorption was closely associated with the deactivation of PXR in CD. Enterohepatic circulation of bile acids is a key factor for preservation of baseline activity of hepatointestinal PXR.

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**Key Words:** bile acids, Crohn's disease, CYP3A4, pregnane X receptor

Inflammatory bowel diseases (IBDs) are chronic inflammatory conditions of the colon and small intestine. The 2 major forms of IBD, ulcerative colitis (UC) and Crohn's disease (CD), are clinically and pathologically overlapping but often very different.[1,2] UC has nontransmural inflammation that is limited to the colon, whereas CD shows transmural inflammation that can involve the entire gastrointestinal tract. The etiology of IBD is still unclear but is thought to result from the interplay of genetic and environment factors.

Recent studies have indicated that dysregulation of the pregnane X receptor (PXR; NR1I2) expression or activity may contribute to the pathophysiology of IBD.[3] PXR, a nuclear receptor, is a member of a family of ligand-activated transcription factors, which regulates detoxification of steroids and xenobiotics and is critical for the maintenance of intestinal integrity. The well-characterized target gene of PXR is cytochrome P450 3A4 (CYP3A4) that is involved in the detoxification of steroids and xenobiotic compounds with broad substrate specificity.[4] Multidrug resistance protein 1 (MDR1; ABCB1), an efflux pump for xenobiotics with broad substrate specificity, seems to be another target of PXR. When PXR is activated by ligand substrates, it binds to response elements in the *CYP3A4* and *MDR1* gene promoters, so that the production of CYP3A4 and MDR1 proteins is upregulated.[5] In addition to the detoxification of steroids and xenobiotics, PXR ameliorates intestinal inflammation through inhibitory effects on both the proinflammatory transcription factor nuclear factor κB[6,7] and the proinflammatory cytokine tumor necrosis factor α (TNF-α).[8,9]

PXR knockout mice show marked mucosal inflammation in the small intestine.[6] In humans, specific polymorphisms in the *PXR* locus that are associated with decreased PXR activities are correlated with an increased susceptibility to IBD.[10,11] Furthermore, the activation of PXR by rifaximin, a nonabsorbable antibiotic, showed therapeutic effects on patients with CD[12] and on the dextran sulfate sodium–induced and trinitrobenzene sulfonic acid–induced IBD model mice.[13] Rifaximin increased detoxification of xenobiotics and antagonized the effects of TNF-α and nuclear factor κB in both intestinal epithelial cells and colon biopsies.[8,9]

Bile acid malabsorption (BAM) is one of the common features of CD with ileitis or ileal resection. Åkerlund et al[14] demonstrated a positive correlation between hepatic activity of

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cholesterol 7α-hydroxylase, the rate-limiting enzyme in the bile acid biosynthetic pathway, and the length of resected ileum. More recently, Lenicek et al[15] have shown that serum levels of 7α-hydroxy-4-cholesten-3-one (C4), a marker for hepatic bile acid biosynthesis,[16] are significantly increased and those of FGF19, a marker of intestinal bile acid flux or farnesoid X receptor (FXR) activity,[17] are significantly decreased in patients with CD with ileal inflammation or resection compared with those in healthy controls. Because bile acids are potential activators of PXR,[18,19] we hypothesized that hepatointestinal PXR activities were downregulated in patients with CD due to BAM followed by reduced enterohepatic circulation of bile acids.

The aim of this study was to explore the relationship between BAM and PXR activities in patients with CD. We measured serum markers of hepatic CYP3A4 activity[20] and those of cholesterol and bile acid metabolism. The results showed that the degree of BAM was closely associated with the deactivation of PXR in patients with CD.

## MATERIALS AND METHODS

### Subjects and Sample Collection

Thirty-one patients with IBD, including 21 with CD and 10 with UC, were studied. Four of the patients with CD had the history of ileal resection, whereas the other 17 patients had no previous surgeries. Among the 17 patients with CD without previous surgery, 16 were the ileum or ileum + colon type and only 1 was the colon type. Patients were diagnosed as UC or CD by clinical, endoscopic, histopathological, and radiological examinations. Detailed baseline characteristics of the patients are described in Table 1. Blood samples were collected from the patients in the morning before breakfast after an overnight fasting, and sera were stored at −20 °C until analysis. Control sera at fasting were obtained from healthy volunteers, and 26 sex-matched and age-matched samples were used in this study. Informed consent was obtained from all subjects, and the experimental protocol was approved by the Ethics Committee of Tokyo Medical University Ibaraki Medical Center.

### Serum Sterol Analysis

Total serum cholesterol concentrations were measured by enzymatic methods using the Cholesterol E-Test Wako (Wako Pure Chemical Industries, Osaka, Japan). Noncholesterol sterols (lathosterol, sitosterol, campesterol, and oxysterols) were quantified by LC-MS/MS as described in our previous article.[21] Briefly, coprostanol and deuterated oxysterols were added to 5 μL of serum as internal standards, and alkaline hydrolysis was carried out in 1 N ethanolic KOH with butylated hydroxytoluene at 37°C for 1 hour. Sterols were extracted with *n*-hexene, derivatized to the picolinyl esters, and injected into the LC-ESI-MS/MS system, a TSQ Vantage triple stage quadrupole mass spectrometer

**TABLE 1.** Baseline Characteristics

| Characteristics | CD | CD-PR | UC | Control |
|---|---|---|---|---|
| Age | 32.3 ± 2.5 | 44.5 ± 9.8 | 45.2 ± 5.5 | 34.9 ± 1.8 |
| Gender (male/female) | 14/3 | 4/0 | 7/3 | 20/6 |
| Location of CD | | | | |
| Ileum | 6 (35%) | 1 (25%) | — | — |
| Ileum and colon | 10 (59%) | 3 (75%) | — | — |
| Colon | 1 (6%) | 0 (0%) | — | — |
| Location of UC | | | | |
| Proctitis | — | — | 2 (20%) | — |
| Left-side colitis | — | — | 3 (30%) | — |
| Pancolitis | — | — | 5 (50%) | — |
| C-reactive protein (mg/dL) | 1.75 ± 0.73 | 1.14 ± 0.80 | 1.51 ± 0.54 | 0.02 ± 0.01 |
| Erythrocyte sedimentation rate (mm/h) | 21.7 ± 6.4 | 23.0 ± 7.2 | 19.8 ± 6.1 | 3.4 ± 1.5 |
| CD Activity Index score | 174 ± 77 | 162 ± 62 | — | — |
| Mayo score | — | — | 4.8 ± 1.2 | — |
| Medications | | | | |
| 5-Aminosalicylates | 17 (100%) | 4 (100%) | 10 (100%) | 0 (0%) |
| Corticosteroids | 4 (24%) | 0 (0%) | 1 (10%) | 0 (0%) |
| Azathioprine | 8 (47%) | 2 (50%) | 5 (50%) | 0 (0%) |
| Infliximab | 9 (53%) | 2 (50%) | 1 (10%) | 0 (0%) |

The numbers of subjects or mean ± standard error of the mean are given. PR, postresection of distal ileum.