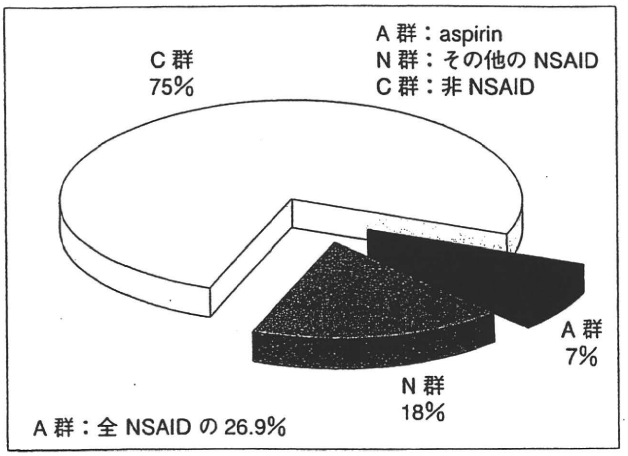


Fig. 1 潰瘍症例の内訳.

2. 方法

A群，N群，C群において平均年齢，性比，基礎疾患，潰瘍既往歴，喫煙，飲酒等の患者背景について検討した.

またA群，N群，C群において臨床症状，内視鏡所見，*HP*感染率(RUT，鏡検，血清抗体を用い，いずれかが陽性ならば陽性，2種類以上で陰性ならば陰性と判定)について検討した.

統計学的検討はMann-Whitney U-test，χ^2-testを用い$p<0.05$にて有意とした.

3. 結果

1) 患者背景

潰瘍症例の内訳はA群25例(7%)，N群68例(18%)，C群289例(75%)であり当院の潰瘍症例の25%で広義のNSAID(L-Aspおよび非aspirin NSAID)を投与されていて7%でL-Aspを投与されていた(**Fig. 1**)．A群のaspirinの投与期間は，1か月以上が22例(88%)と大半で，aspirinの種類は81 mg緩衝aspirinが20例(80%)を占めた．またA群の基礎疾患は虚血性心疾患が11例(44%)，脳血管障害が10例(40%)とほぼ半数ずつであった．N群の基礎疾患は整形外科疾患が37例(54.4%)，リウマチ性疾患が11例(16.2%)と多かった．平均年齢はA群67.7±14.3歳，N群65.1±14.9歳，C群55.3±14.8歳とA群，N群はC群と比し有意に平均年齢が高かった($p<0.0001$)．性比(M/F)はA群18/7，N群33/35，C群216/73とN群で女性が多かった($p<0.0001$)．潰瘍既往歴はA群4例(16%)，N群15例(22%)，C群66例(22.8%)と各群間で有意差は認めなかった．喫煙率はA群11例(44%)，N群31例(45.6%)，C群173例(59.9%)とA群，N群と比しC群で高い傾向を示した($p<0.05$)．また飲酒率は同様にA群9例(36%)，N群19例(27.9%)，C群162例(56.1%)とA群，N群と比しC群で高い傾向を示した($p<0.001$)．

2) 症状

症状の検討(**Fig. 2**)では出血症状(吐血，下血，貧血)の割合はA群53.6%，N群45.2%と広義のNSAID群ではC群の31.2%と比し有意に高率であった($p=0.0052$)．

3) 内視鏡像

潰瘍発生部位の検討では幽門前庭部潰瘍の比率はA群24%，N群32%と両群間で有意差は認められずA群では胃体部の発症も31%に認められた(**Fig. 3**)．潰瘍の大きさの検討では10 mm以下の潰瘍はA群，N群，C群でそれぞれ92%，63.2%，87.9%とA群はN群と比較し10 mm以下の小型の潰瘍が多い結果であった($p=0.0112$)．また潰瘍の数は多発性，単発性の割合で検討したが3群間で有意差は認めなかった.

4) *HP*感染率

広義のNSAID(N群+A群)ではC群と比較し*HP*感染率は低率であったがA群単独ではC群とで*HP*感染率に有意差は認められなかった($p=0.0056$)(**Fig. 4**)．

内視鏡像

当院で経験したNSAID関連上部消化管病変を，非aspirin NSAID関連病変とaspirin関連病変とに分類し呈示する.

1. 非aspirin NSAIDs関連病変

〔**症例1**〕 68歳，女性で当院膠原病内科でRAの加療中の症例．10年前から抗リウマチ薬で内服加療を受けていた．またNSAIDとして数年間diclofenac(25 mg)の内服薬を長期投与されていた．心窩部痛精査で上部消化管内視鏡検査を施行したところ，十二指腸球部前壁に潰瘍性病変を認めた(**Fig. 5 a**)．NSAID長期服用症例であり，NSAID起因性十二指腸潰瘍と診断した．RAに

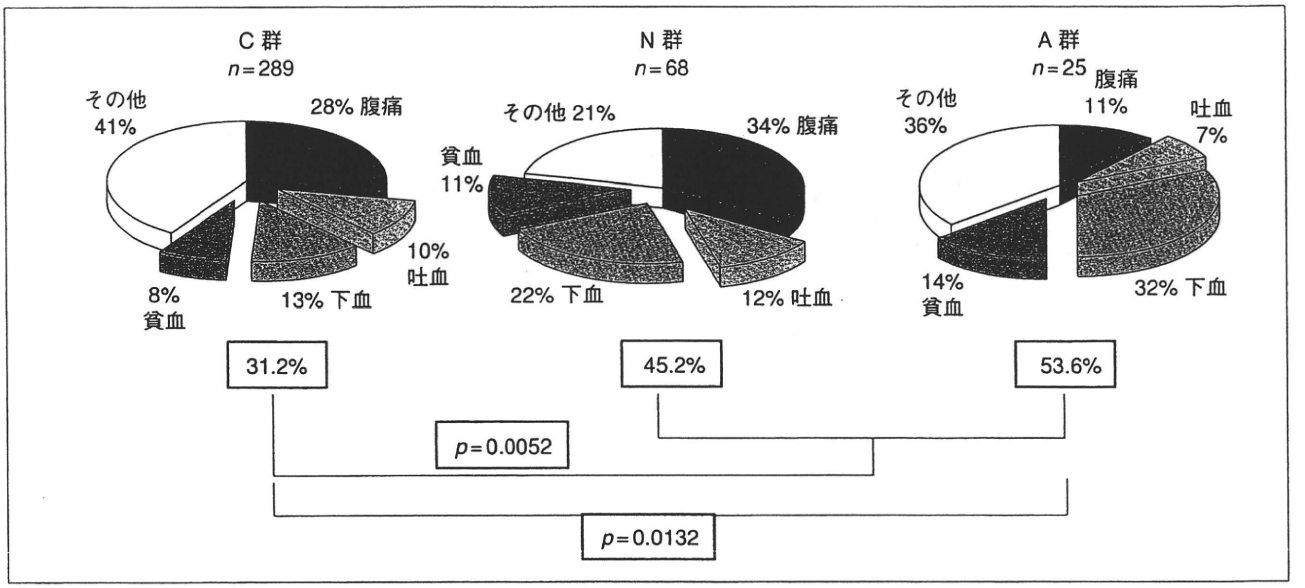


Fig. 2 各群の症状.

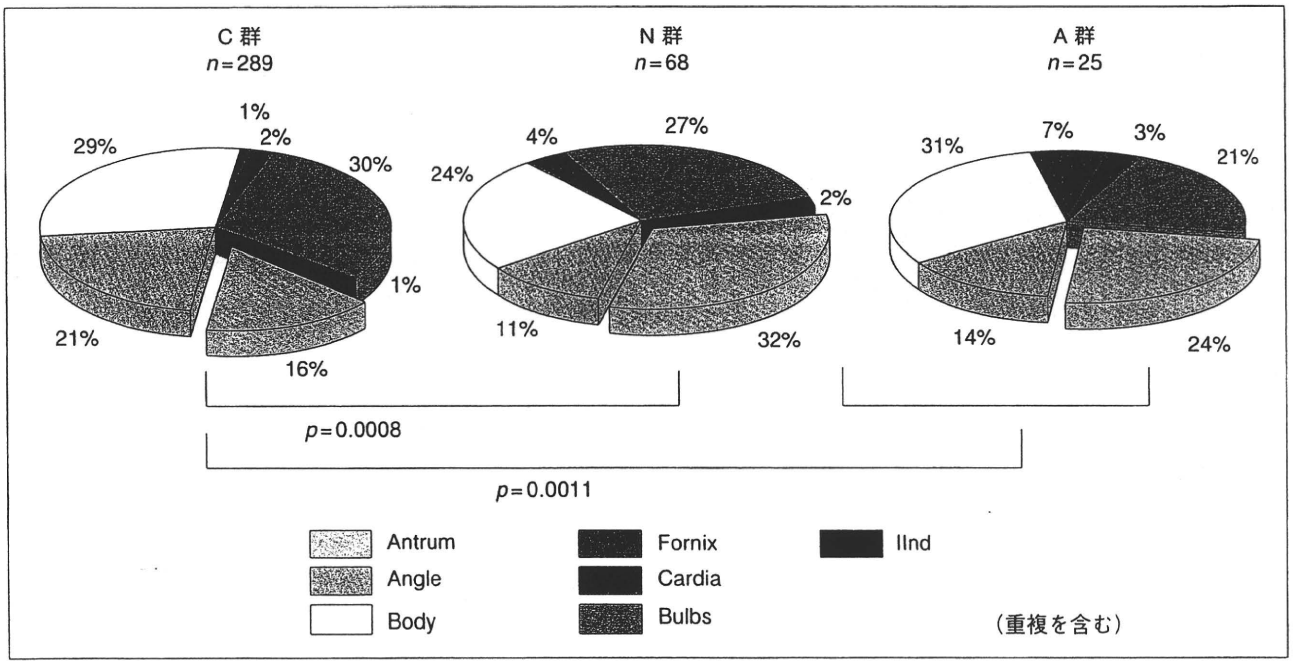


Fig. 3 各群における潰瘍発生部位.

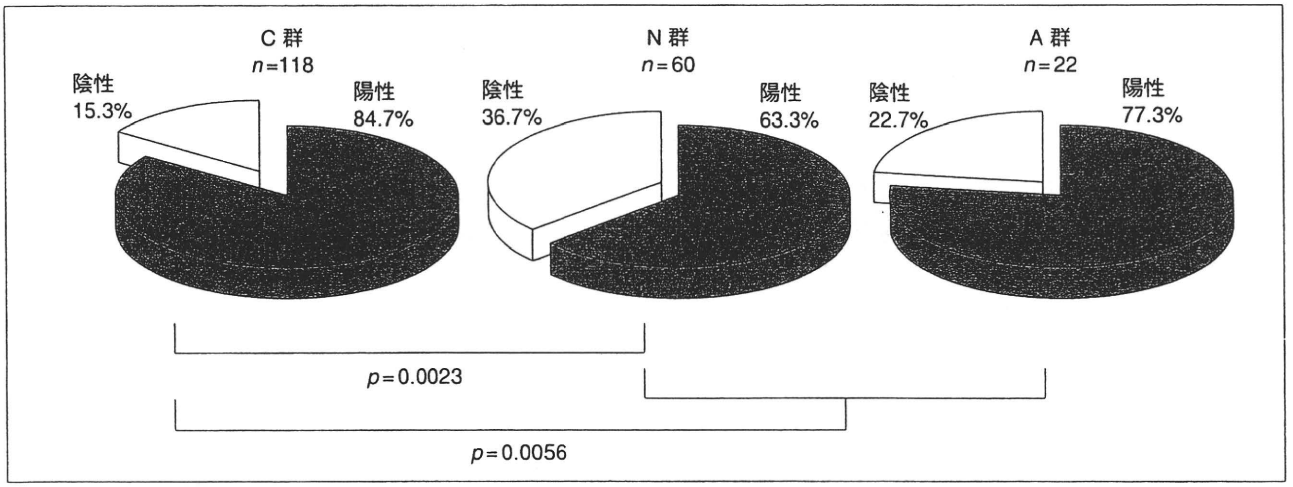
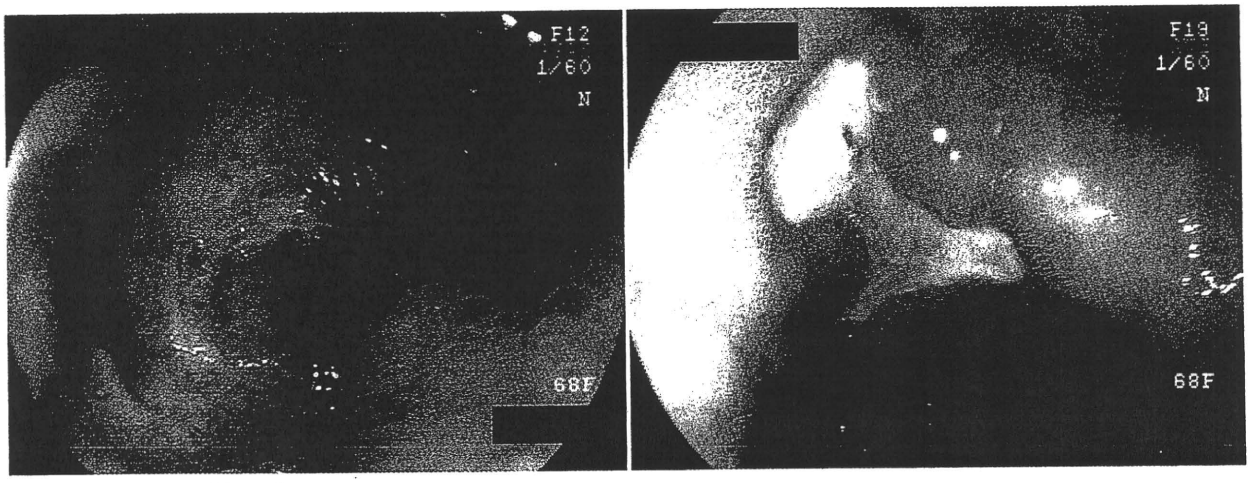
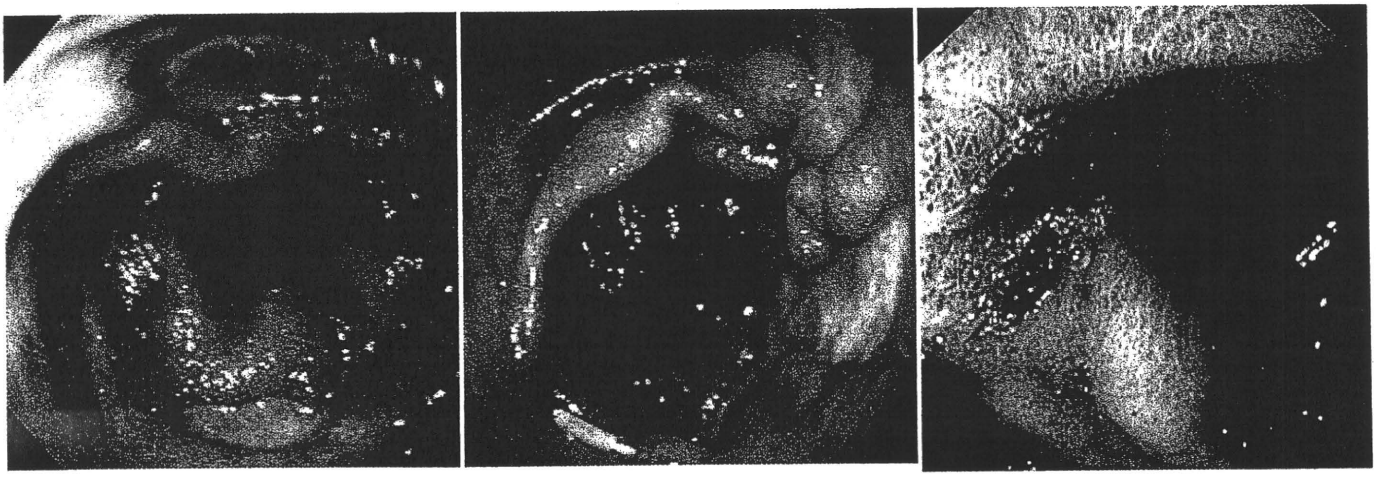


Fig. 4 各群の *Helicobacter pylori* 感染率.



a | b **Fig. 5〔症例 1〕**
a 十二指腸球部前壁の約 2 cm 大の深掘れ潰瘍.
b 胃前庭部小彎の不整形の潰瘍および幽門輪周囲の多発性小潰瘍.

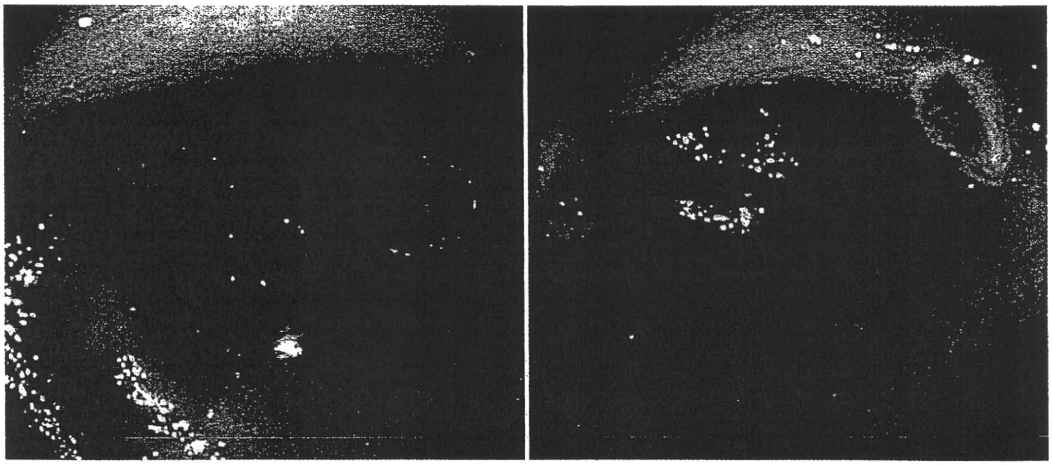


a | b | c **Fig. 6〔症例 2〕**
a, b 胃前庭部に多発する不整形潰瘍.
c 胃前庭部に再発した潰瘍病変.

よる疼痛が強く NSAID 中止困難であったため NSAID は使用頻度を減らし継続とし，同時に lansoprazole 30 mg を開始した．PPI (proton pump inhibitor) 開始後十二指腸潰瘍は改善傾向を示した．本症例は *HP* 陽性であり，NSAID 潰瘍のため *HP* 除菌は慎重を要すると考えたが，十分なインフォームド・コンセントのもと *HP* 除菌を行った．除菌後心窩部痛が再度出現し，上部消化管内視鏡再検施行したところ PPI 継続していたにもかかわらず，胃前庭部に潰瘍の再発を認めた (**Fig. 5 b**)．その後 PG (prostaglandin) 製剤を開始して加療した．

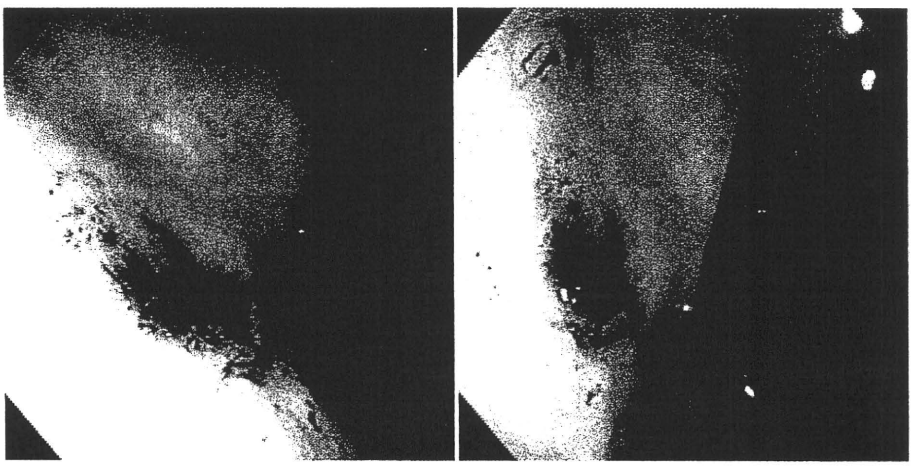
〔**症例 2**〕 53 歳，男性．慢性腎不全で血液透析中の症例．腰椎椎間板ヘルニアがあり，約 3 年間 diclofenac (25 mg) を長期内服していた．心窩部痛を認め上部消化管内視鏡施行したところ，胃前庭部に不整形の潰瘍性病変を認めた (**Fig. 6 a, b**)．一部出血もありクリッピングも施行している．NSAID 長期投与中で発生部位や形態から NSAID 起因性胃潰瘍と診断した．腰痛が著しく NSAID 中止が困難なため，PPI 併用した．PPI 開始後心窩部痛は改善傾向を示したが，3 か月後再度心窩部痛が出現したため上部消化管内視鏡再検したところ，不整形の潰瘍性病変が改善しておらず (**Fig. 6 c**)，NSAID を中止となった．

〔**症例 3**〕 腰痛に対して，整形外科で diclofenac (25 mg) を処方され，数年前から on demand で内服していた．心窩部痛等の症状は認め



a | b

Fig. 7〔**症例 3**〕
a 幽門輪付近の潰瘍病変.
b 胃角小彎にも多発する潰瘍病変.



a | b

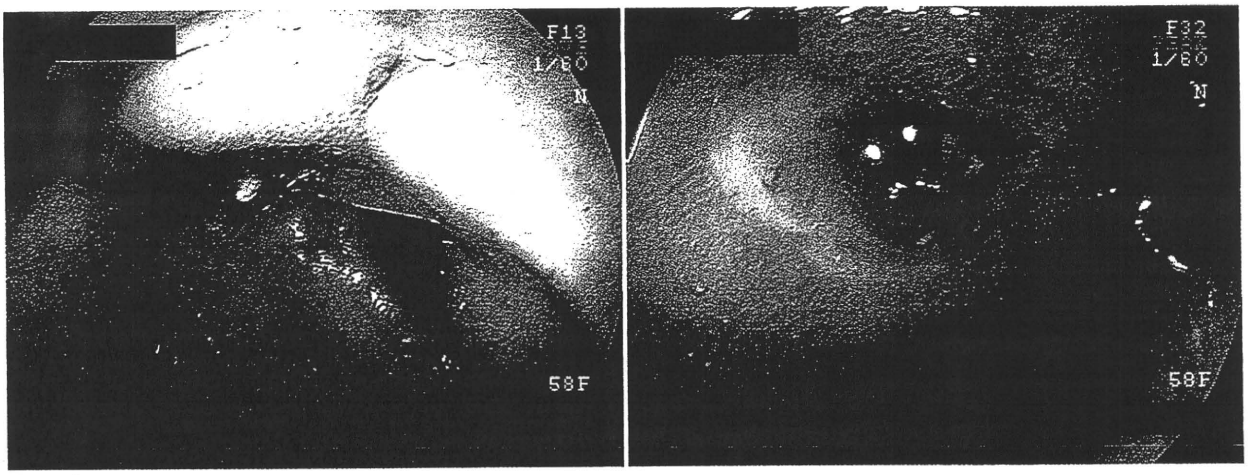
Fig. 8〔**症例 4**〕
a 胃体上部小彎の比較的浅い出血性潰瘍病変.
b 潰瘍は比較的浅いが露出血管が目立つ.

なかったが，Hb 7.8 g/d*l* と著明な貧血を指摘され上部消化管精査の依頼があった．上部消化管内視鏡検査を施行したところ，幽門前庭部に不整形の潰瘍性病変を認め(**Fig. 7 a**)，また胃角部にも比較的浅い割には血管の露出を伴う潰瘍が 2 か所認められた(**Fig. 7 b**)．

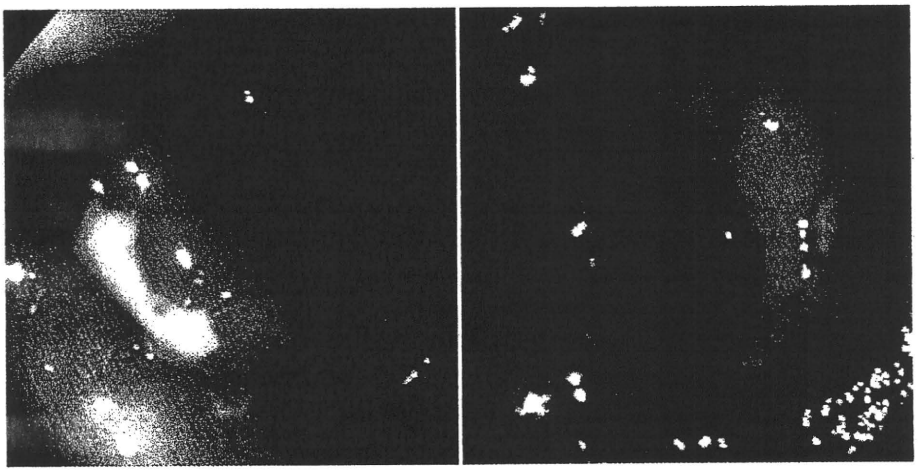
〔**症例 4**〕 84 歳，男性．変形性腰椎症に対して，約 1 年前から loxoprofen を内服していた．吐血を主訴に救急外来受診し，上部消化管内視鏡検査施行し，胃体上部小彎に約 10 mm 大の比較的浅い潰瘍性病変を認め，出血を認めた．明らかな露出血管が認められ，エタノール注入を施行し止血に成功した(**Fig. 8 a, b**)．

〔**症例 5**〕 58 歳，女性．肺癌の疼痛に対して diclofenac(25 mg)坐薬を約 1 年前から頓用で 1 日 2〜3 個使用していた．ふらつき等の貧血症状とタール便が出現し，Hb 7.7 g/d*l* と著明な貧血を認めた．上部消化管内視鏡検査施行したところ，胃前庭部小彎に約 5 mm 大と比較的小さい潰瘍を認め，潰瘍底に太い露出血管を伴っていた(**Fig. 9 a, b**)．露出血管に対しクリッピングを施行し検査を終了した．

〔**症例 6**〕 74 歳，男性．整形外科にて，腰部脊柱管狭窄症と診断され 2 年前から diclofenac SR を内服中の症例．心窩部痛を主訴に上部消化管内視鏡検査施行したところ，胃前庭部大彎に約 10 mm 大の潰瘍と小型の潰瘍，びらんが散在していた(**Fig. 10 a, b**)．



a | b **Fig. 9〔症例 5〕**
a 胃前庭部小彎の露出血管を伴う出血性潰瘍病変.
b 近接すると潰瘍の大きさの割には太い露出血管を有していた.



a | b **Fig. 10〔症例 6〕**
a 幽門輪付近の不整形潰瘍病変.
b 前庭部に浅い潰瘍病変が多発する.

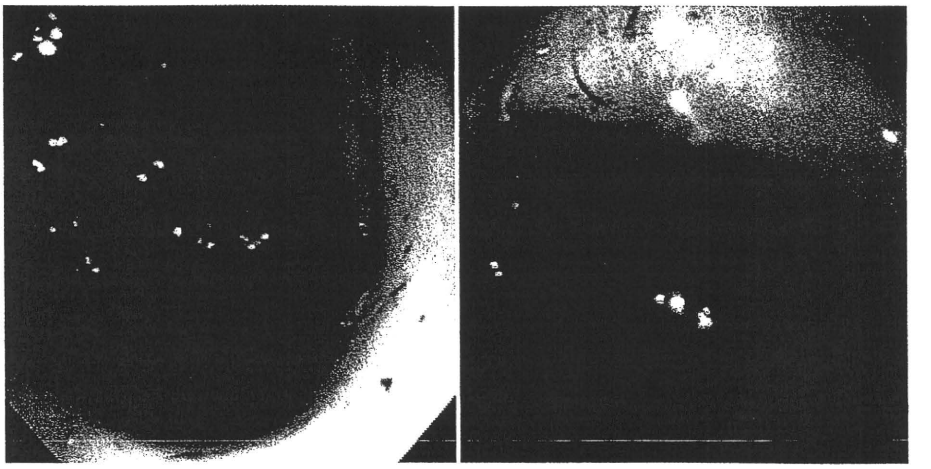
2. aspirin 関連病変

〔症例 7〕 71 歳，男性．脳梗塞の既往歴があり，数年前から aspirin 81 mg を内服中であった．スクリーニング目的の上部消化管内視鏡検査を施行したところ，胃前庭部から胃角部にかけて，径 2～3 mm 大の小型の浅い潰瘍性病変および発赤びらんが散在していた(**Fig. 11 a, b**)．

〔症例 8〕 54 歳，男性．虚血性心疾患に対して，数年前から aspirin 81 mg を内服中であった．タール便が出現し，上部消化管内視鏡検査を施行したところ十二指腸球部から下行脚への移行部付近に比較的深い潰瘍性病変とそのほかびらん性病変が散在していた(**Fig. 12 a, b**)．

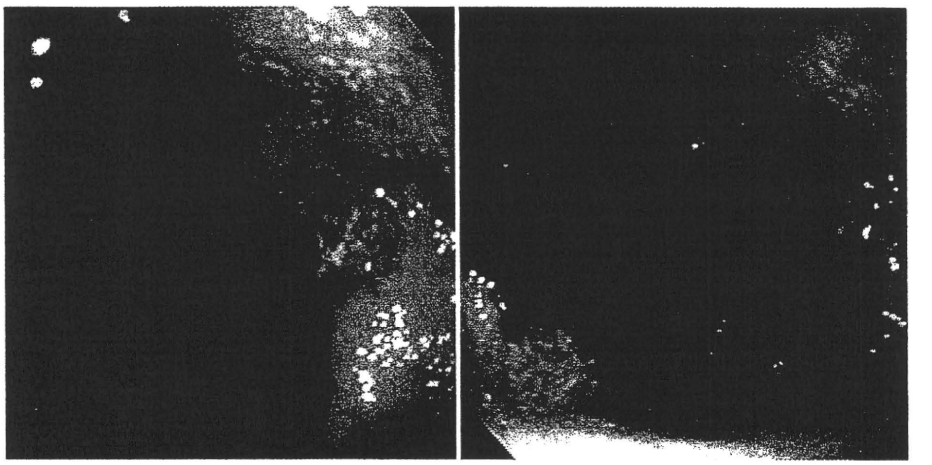
〔症例 9〕 69 歳，女性．脳梗塞の既往歴があり数年前から aspirin 81 mg を内服中であった．心窩部痛のため上部消化管内視鏡検査を施行したところ，胃体部大彎に縦走性で細長い形態を呈する潰瘍性病変を認めた(**Fig. 13 a, b**)．

〔症例 10〕 64 歳，男性．虚血性心疾患に対して，数年前から aspirin 81 mg を内服中であった．心窩部痛は認められなかったが，タール便が出現し上部消化管内視鏡検査を施行したところ，胃角小彎前壁に径 4 mm 大と小型で浅いが出血を伴う潰瘍性病変を認めた(**Fig. 14 a, b**)．アルゴンプラズマ凝固法 (algon plasma coagulation ; APC) を用いて止血した．



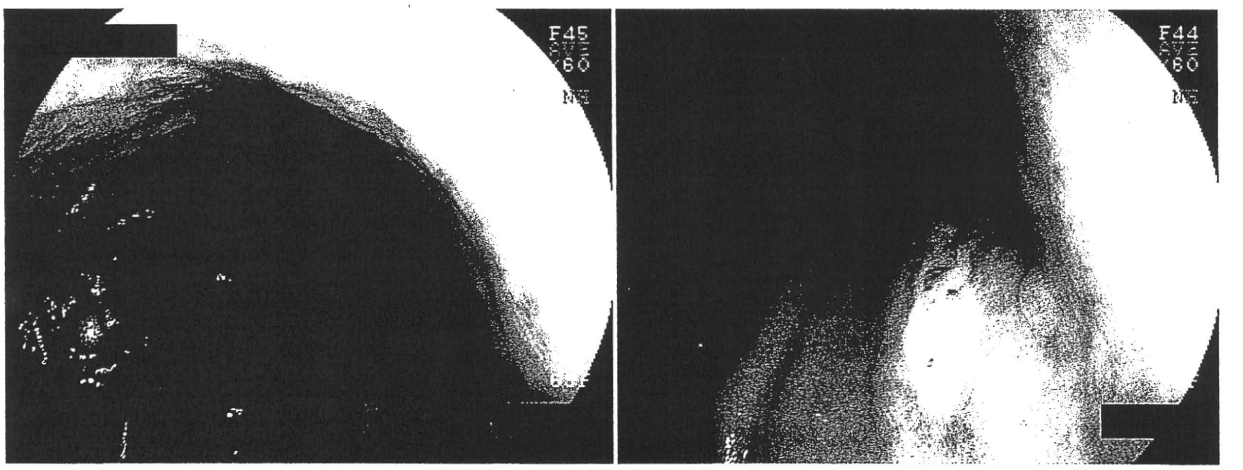
a | b

Fig. 11〔症例 7〕
a 前庭部に縦走傾向を示す，浅いびらん性病変が散在する．
b びらん性病変は胃角小彎まで散在する．



a | b

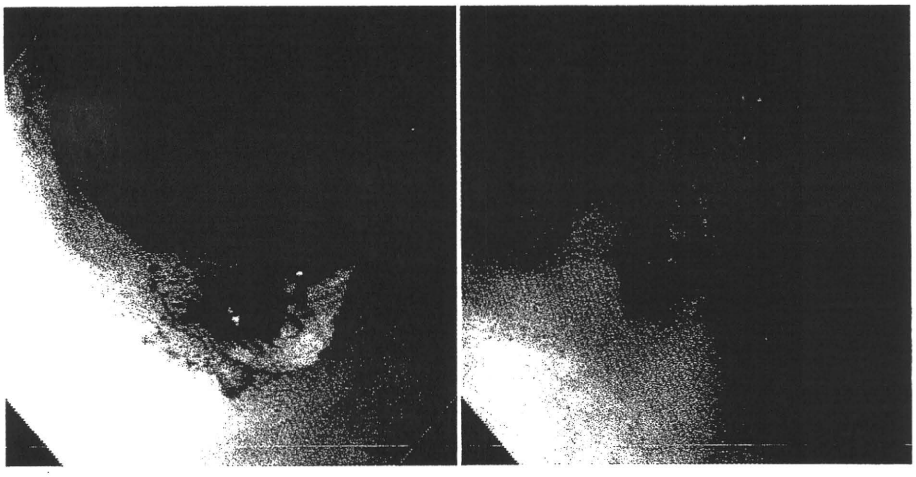
Fig. 12 a, b〔症例 8〕 十二指腸球部の前壁よりに深い潰瘍病変を認め，その肛門側に霜ふり様のびらん性病変が散在する．



a | b **Fig. 13 a, b〔症例 9〕** 胃体上部大彎に細長く，比較的浅い形態を示す潰瘍性病変を認める．

考　察

NSAID は薬物性潰瘍の中では最も頻度が高いことが，20 年以上の前から指摘されている．従来，整形外科疾患，リウマチ性疾患で処方されている NSAID による消化管粘膜傷害が問題となっている．粒良ら[4]は膠原病およびその類似疾患に対し，ステロイドおよび NSAID の長期投与中に認められた胃潰瘍について，非投与の胃潰瘍と比較検討し，前庭部に好発するなどの特徴についてまとめている．溝上ら[2]は NSAID 服用中の RA 患者 85 症例を検討し，胃潰瘍は 35％，十二指腸



a | b

Fig. 14 a, b〔症例 10〕 胃角小彎前壁に比較的浅く露出血管を伴う潰瘍性病変を認めた.

Table 1 低用量 aspirin による消化管出血のリスク

消化管出血の発生のオッズ比 (aspirin 量 50〜162.5 mg/日)			
Study	aspirin	対照	オッズ比(95 %CI)
Diener, et al.	25/ 1,649	19/ 1,649	1.32(0.73〜 2.39)
Hansson, et al.	107/ 9,399	55/ 9,391	1.91(1.40〜 2.60)
Petersen, et al.	1/ 336	0/ 336	7.39(0.15〜372.41)
SALT	11/ 676	4/ 684	2.60(0.94〜 7.19)
TPT	22/ 1,268	10/ 1,272	2.14(1.07〜 4.30)
Wallentin	0/ 399	0/ 397	
Silagy, et al.	6/ 200	0/ 200	7.58(1.51〜 37.93)
USPHS	402/11,037	274/11,034	1.48(1.27〜 1.72)
Total	574/24,964	362/24,963	1.59(1.40〜 1.81)

0.1 0.2 1 5 10
aspirin が優れる 対照が優れる

(文献 3 より改変)

潰瘍は 8 % で認められたと報告した. 日本リウマチ財団の疫学調査[3]が最も大規模な検討であるが, NSAID を 3 か月以上服用している長期投与中の RA 患者では 15.5 % に活動性の胃潰瘍を認め, 1.9 % に十二指腸潰瘍を認めたと報告した. また NSAID 起因性潰瘍が消化性潰瘍全体に占める割合に関しては, 菅野[5]は 13 % が NSAID 関連潰瘍であると指摘しており, われわれの検討では広義の NSAID (N 群と A 群) が潰瘍全体の約 25 % を占めていた (Fig. 1). 出血性潰瘍における NSAID 潰瘍の占める割合についても検討されており, 出血性潰瘍の 24 % が NSAID 関連潰瘍で, NSAID 関連潰瘍のうち 27 % が aspirin 関連であったと報告している[6].

aspirin は最も古くから全世界で使用されている代表的な NSAID であるが, 約 20 年前ごろからわが国でも虚血性心疾患や虚血性脳疾患の二次予防として処方されている. 低用量 aspirin による上部消化管傷害も少ないながら報告されている[7]. aspirin による出血性潰瘍について, 無作為化比較試験が施行された 24 試験のメタアナリシスの結果によると発生頻度は 2.47 % (プラセボ投与では 1.68 %), オッズ比は 1.68 (95 % CI : 1.51〜1.88) であり潰瘍出血のリスクとなると報告している (**Table 1**)[8]. また aspirin 製剤の素剤, 腸溶剤, 制酸緩衝剤などの剤形の違いでの上部消化管傷害についての検討も行われているが, 剤形の違いによる消化管傷害の違いはないと指摘している[9]. Cryer ら[10]は, ボランティアに 3 か月間 aspirin を投与した結果から胃粘膜傷害は 1 日用量

10 mg 以下でも発生すると報告している．L-Asp による上部消化管出血のリスクを増加させる因子として，潰瘍の既往[11]，NSAID の併用[12]が挙げられており，一方減少させる因子としては亜硝酸剤の併用[13]や H_2 ブロッカー，PPI などの酸分泌抑制剤の併用[11]が挙げられている．

粘膜傷害の軽減が期待される選択的 COX-2 阻害薬は，2000 年の rofecoxib を用いた VIGOR (Vioxx Gastrointestinal Outcomes Research) 試験[14]や celecoxib を用いた CLASS (Celecoxib Long-term Arthritis Safety Study) 試験[15]での結果はすばらしいものであったが，副作用として心血管傷害の発生が報告[16]され，2004 年 10 月以降米国において市場からの撤退が相次いでいる．

NSAID 潰瘍の臨床症状についての検討では腹痛等の腹部症状が乏しく，出血症状で発症することが従来から指摘されている．溝上ら[17]は NSAID 長期投与 RA 患者の胃潰瘍症例 69 例を対象に臨床症状を検討し，腹部症状を欠き進行性の貧血のみを呈する例が 44.9％と高率であったと報告した．また塩川ら[3]の日本リウマチ財団の潰瘍症例の検討では NSAID 起因性潰瘍の 42％が無症状であると報告している．また中村ら[18]は NSAID 起因性潰瘍入院例の 75％が吐下血等の出血症状を主訴としているとの指摘をしている．今回のわれわれの検討でも N 群でコントロール群と比し，出血症状が多いという同様の結果であった (Fig. 2)．また L-Asp 潰瘍の臨床症状に関する報告は少ないが，今回のわれわれの検討からは L-Asp 潰瘍でも NSAID 起因性潰瘍同様に腹部症状に乏しく出血症状で発症する症例が多く認められた (Fig. 2)．

潰瘍発生部位に関しては，従来の報告では NSAID 潰瘍は NSAID 投与期間により好発部位は異なることが指摘されており，長期投与では前庭部の潰瘍が多く短期投与での急性発症の潰瘍は胃体部が多いと言われている[4][18]．われわれの検討ではコントロール群と比し N 群と A 群ではいずれも前庭部の潰瘍が多く認められ，特に N 群では前庭部の潰瘍が占める割合が多かった．また A 群では N 群と比し長期投与でも比較的胃体部の症例が多い傾向を示した (Fig. 3)．潰瘍の大きさの検討では 10 mm 以下の潰瘍は A 群，N 群，C 群でそれぞれ 92％，63.2％，87.9％と L-Asp 潰瘍では非 aspirin NSAID 潰瘍と比し小型の潰瘍が多いという結果であった ($p = 0.0112$)．また潰瘍の個数に関しては L-Asp 潰瘍と非 aspirin NSAID 潰瘍で有意な差は認められなかった．形態に関しては，通常の潰瘍と比し，不整形の潰瘍が多いことが指摘されている[19][20]．低用量 aspirin 起因性と非 aspirin NSAIDs 潰瘍での潰瘍発生部位や大きさ等の形態に関する検討は少ないため今後症例の蓄積が望まれる．

NSAID による上部消化管病変の内訳に関する検討も行われている．リウマチ財団の検討[3]では上部消化管病変の総有病率が報告されている．1,008 例中，上部消化管に異常ありが 627 例 (62.2％) で，内訳は胃潰瘍 156 例 (15.5％)，十二指腸潰瘍 19 例 (1.9％)，胃炎 388 例 (38.5％)，十二指腸炎 27 例 (2.7％)，AGML (acute gastric mucosal lesion) 6 例 (0.6％)，食道病変 24 例 (2.4％) であったと報告している．この報告では胃炎の発現部位は前庭部が 77.8％でまた小彎側が 70.6％と圧倒的に多く，重症度は軽度のものが多かった．また AGML は前庭部と胃角部に多く，十二指腸炎は球部が 96.3％と圧倒的に多かったと報告している．

当院の検討では広義の NSAID 群 (A 群＋N 群) での *HP* 陽性率は低率であったが A 群単独では C 群と有意差は認めなかった．また Mizokami ら[21]は NSAID 長期投与例では *HP* 陽性率が低下することを報告している．NSAID と *HP* はともに潰瘍の 2 大原因とされている．しかし両者の傷害機序は異なっており，独立した因子とされている．NSAID と *HP* 感染との相互作用については，相反する結果が報告されている[22][23]．しかし Huang ら[24]は多数の論文からのメタアナリシスの結果を報告しており，NSAID 使用者での *HP* 陽性例では潰瘍発生率のオッズ比が 3.53 (95％ CI；2.16～5.75) であり，*HP* 陽性例は陰性例に比べ約 3.5 倍高率に潰瘍が発生するとしており，臨床的には *HP* 感染は NSAID 潰瘍の発生率を増加させると指摘している．本邦での大規模疫学調査[25]でも上部消化管出血のリスクは NSAID (－)

HP 感染(−)と比し NSAID(−)*HP* 感染(+)では 5.4 倍，NSAID(+)*HP* 感染(−)では 4.9 倍，NSAID(+)*HP* 感染(+)では約 10 倍であると報告されており，NSAID と *HP* 感染は相加的に作用すると考えられる．一方 L-Asp と *HP* の相互作用に関する検討は少ないが，Chan ら[26]は消化管出血症例を対象として再出血のリスクを検討している．aspirin 投与症例では *HP* 除菌により再出血が予防されたと報告し，aspirin 投与例における *HP* 除菌の有効性を指摘している．

L-Asp 起因性潰瘍の治療や再発予防に関する一定の見解は得られていないが，最近「胃潰瘍診療ガイドライン」[27]で L-Asp 投与下での消化性潰瘍再発防止には，*HP* 除菌療法は単独では効果が不十分であり，PPI の投与が奨励されている．ただし，本邦でのデータが少なく今後症例を蓄積していく必要がある．

おわりに

従来から指摘されている，非 aspirin NSAID の長期投与に典型的な幽門前庭部に発症する胃潰瘍は減少傾向にある．一方，虚血性心疾患，脳血管障害が増加しており，二次予防として L-Asp の処方が急増しており，L-Asp に起因する胃体部の潰瘍が増加している．L-Asp に起因する胃体部出血性潰瘍は止血困難例も多く，今後潰瘍の予防が重要になると考える．

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Summary

Clinical Features and Endoscopic Findings of NSAID-related Mucosal Lesions in the Upper Gastrointestinal Tract

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It has been demonstrated that Non-steroidal anti-inflammatory drugs (NSAID) have been the most common cause of drug-induced ulceration in the last 20 years. With the advent of our aging society, the number of patients with ischemic heart disease and cerebrovascular disease has increased. This has resulted in the widespread use of Low-dose aspirin (L-Asp). It is suggested that L-Asp induces gastrointestinal mucosal injury. In this study, we classified peptic ulcers diagnosed in our hospital into the low-dose aspirin-induced group (Group A), the non-aspirin-NSAID-induced group (Group N), aspirin and non-aspirin-NSAID free group (Group C). The proportion of Group N and Group A was 25% and 7%. Bleeding events such as hematemesis, melena and anemia occurred in 53.6%, 45.2% and 31.2% in Group A, N and C. The ratio of ulcers located in the antrum were 24%, 32% and 16% in Group A, N and C. The ratio of ulcers located in the antrum was higher in Group A and N than in Group C. There was a tendency for the size of ulcers to be smaller in Group A than in Group N. There was no difference in the number of ulcers in each group. The prevalence of *H. pylori* was significantly lower in group N than in Group C. There was no diffrence in the prevalence of *H. pylori* in Group A and C.

It has been suggested that typical NSAID-induced ulcers have decreased and on the other hand, L-Asp-related ulcers located in the corpus have increased. Prevention of L-Asp-related gastrointestinal mucosal damage is needed.

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Review Article

Ursodeoxycholic acid: Mechanism of action and novel clinical applications

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Ursodeoxycholic acid (UDCA) is used in the treatment of cholestatic liver diseases, gallstone dissolution, and for patients with hepatitis C virus infection to ameliorate elevated alanine aminotransferase levels. The efficacy of UDCA treatment has been debated and the mechanisms of action in humans have still not defined. Suggested mechanisms include the improvement of bile acid transport and/or detoxification, cytoprotection, and anti-apoptotic effects. In this review, we summarize the proposed molecular mechanisms for the action of UDCA, especially in hepatocytes, and also discuss the putative future clinical usage of this unique drug.

Key words: bile acids, cholestatic liver diseases, colon cancer, non-alcoholic steatohepatitis, ursodeoxycholic acid

INTRODUCTION

BILE ACID SYNTHESIS from cholesterol is the primary pathway for cholesterol catabolism. Bile acids are amphipathic molecules that contain a sterol nucleus with hydroxyl groups and a side chain with a terminal carboxylic acid. Their amphipathic nature is essential for the solubilization of dietary lipids, which subsequently promotes lipid absorption in the digestive tract. The principal bile acid in humans are cholic acid (CA) and chenodeoxycholic acid (CDCA), which are primary bile acids, and deoxycholic acid (DCA) and lithocholic acid (LCA), which are secondary bile acids, and their glycine and taurine conjugates. Most bile acids are present in the enterohepatic circulation and are stored in the gallbladder. When a meal is ingested, bile acids flow into the duodenum and intestine, and are efficiently re-absorbed by passive diffusion and active transport in the terminal ileum and transported back to the liver via the portal vein. In the liver, the bile acids are taken up at the sinusoidal (basolateral) membrane and exported at the canalicular (apical) membrane of hepatocytes into bile canaliculus. Each bile acid molecule completes 4–12 cycles between the liver and intestine per day. Because of this efficient recirculation system, approximately only 5% of the bile acid pool is derived from de novo biosynthesis in the liver.

Ursodeoxycholic acid (UDCA) is a bile acid that is present in human bile at low concentrations, as only 3% of total bile acids. UDCA is a 7,-hydroxy epimer of the primary bile acid CDCA (Fig. 1), and can be isolated from the Chinese medicine Yutan, which is derived from the dried bile of adult Chinese black bears. UDCA has been used widely in clinical applications in the Western world since the mid 1980s, and has been shown to improve clinical and biochemical indices in a variety of biliary and liver diseases (Table 1). In the 1970s, the first prospective study of UCDA for the treatment of patients with gallbladder stones demonstrated gallstone dissolution,[1] and it is now recognized that UDCA dissolves gallstones by solubilizing cholesterol from the stone surface. UDCA also converts supersaturated bile to unsaturated bile,[2] and such desaturation enhances the transport capacity of bile for cholesterol.[2] Biliary desaturation by UDCA occurs through several mechanisms, most of which are not completely understood.[2] In addition, UDCA also has the unique property of promoting the formation of a liquid crystal mesophase of phospholipids and cholesterol.[3] Such liquid crystals can form even in the presence of bile supersaturated with cholesterol, which may account for the observation that UDCA can dissolve gallstones in the presence of supersaturated bile.

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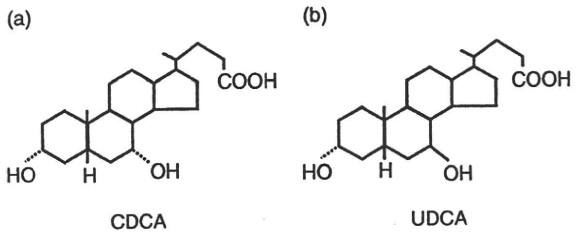

Figure 1 Chemical structure of (a) chenodeoxycholic acid (CDCA) and (b) ursodeoxycholic acid (UDCA). UDCA is a 7,-hydroxy epimer of the primary bile acid CDCA. Because of the different planar orientation of the 7-hydroxy group, UDCA is more hydrophilic than CDCA.

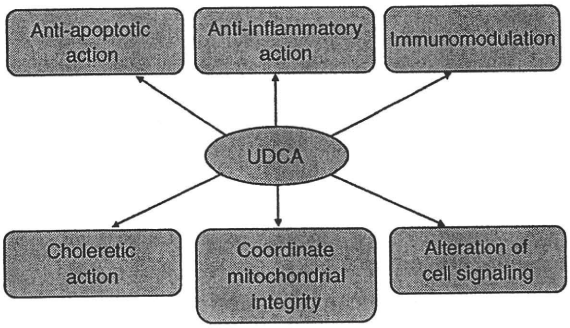

Figure 2 Putative mechanism of action of ursodeoxycholic acid (UDCA). UDCA exerts its action(s) in the liver through multiple mechanisms that are possibly interrelated with each other. Despite frequent novel discoveries of the molecular functions of bile acids, clear evidence for the mechanism of UDCA has yet to emerge.

It has been claimed that UDCA reduces the risk of biliary pain, regardless of gallstone dissolution.[4] The proposed underlying mechanisms for this effect are impaired gallbladder motility with increased fasting and residual postprandial gallbladder volumes,[5] less cholesterol crystals,[6] or decreased mucin contents in bile[7] upon UDCA treatment. We analyzed the effect of UDCA (600 mg/dL) in a cohort of 527 uncomplicated gallstone patients who were followed for up to 18 years.[8] UDCA therapy was associated with a reduced risk of biliary pain in both symptomatic (62% *vs* 92% in untreated patients at 10 years, $P < 0.001$, relative risk: 0.19; 95% Cumulative Index (CI), 0.10–0.34) and asymptomatic patients (6% *vs* 12% in untreated patients at 10 years, $P = 0.037$, relative risk: 0.19; 95% CI, 0.04–0.91). The risk for conversion to cholecystectomy due to frequent attacks or cholecystitis was also reduced in UDCA-treated symptomatic patients (26% *vs* 88% in untreated patients at 10 years, $P < 0.001$, relative risk: 0.08; 95% CI, 0.03–0.22). These effects were independent of stone dissolution. Although a recent placebo-controlled study suggested that UDCA does not reduce symptoms in highly symptomatic patients,[9] it still appears worthwhile to consider UDCA for patients with mild to moderate symptomatic gallstones.

Table 1 Current clinical use of ursodeoxycholic acid

Gallstone dissolution[1]
Prevention of biliary pain[8]
Adult cholestatic liver diseases
Primary biliary cirrhosis[11]
Primary sclerosing cholangitis[77]
Intrahepatic cholestasis of pregnancy[78]
Pediatric cholestatic liver diseases
Cystic fibrosis[79]
Progressive familial intrahepatic cholestasis[80]
Drug-induced cholestasis
Chronic viral hepatitis (ameliorate the elevation of alanine aminotransferase levels; no effect upon viral load)[31]

Besides the evaluation of UDCA as a gallstone-dissolving agent, the beneficial effects of UDCA in patients with hepatic disorders were reported in the 1980s. UDCA improves clinical and biochemical serum parameters in a variety of cholestatic diseases,[10] and is now considered as the first-line treatment for patients with chronic cholestatic liver diseases, such as primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), intrahepatic cholestasis of pregnancy (ICP), and several less common adult and pediatric cholestatic conditions. For PBC, UDCA is the only drug approved by the Food and Drug Administration (FDA) for the treatment of the disease before liver transplantation. A combined analysis of the three largest randomized clinical trials of UDCA for PBC indicated that UDCA improves survival without liver transplantation.[11] However, the efficacy of UDCA treatment has been debated[12,13] and the mechanisms of action in humans are still not defined,[10] even 70 years after its initial isolation by a Japanese scientist. The suggested mechanisms of UDCA include improved bile acid transport and detoxification, cytoprotection, and anti-apoptotic effects (Fig. 2). In the present review, we focus on the unique character of UDCA by discussing what is known about these mechanisms, and we suggest possible novel clinical applications of UDCA.

PROPOSED MOLECULAR MECHANISMS FOR THE ACTION OF UDCA

Choleretic effect of UDCA

IN PATIENTS WITH cholestasis, hydrophobic bile acids accumulate in hepatocytes and can cause cell damage, apoptosis, and necrosis. In experimental cholestasis, UDCA stimulates biliary secretion of bile acids, bilirubin glucuronides, glutathione conjugates, and other organic anions. In the rodent liver, UDCA counteracts cholestasis induced by hydrophobic bile acids by transcriptional and post-transcriptional mechanisms.[14] In patients with PBC and primary PSC, UDCA stimulates biliary secretion of bile acids,[15] and during long-term treatment, decreases the elevated serum levels of bilirubin and CDCA.[10] Therefore, UDCA might exert beneficial effects in cholestatic disease; in part, by stimulating the elimination of toxic compounds from hepatocytes. The secretion capacity of hepatocytes is determined by the number and activity of transporter proteins in the basolateral membrane, and crucially, the canalicular membrane. The expression of transporter proteins is regulated at the transcriptional and post-transcriptional levels,[14] since UDCA stimulates the expression of transporters required for biliary secretion in the liver of humans[16,17] and in experimental models[17,18] (Fig. 3), and stimulates the targeting and insertion of transporters into the hepatocyte canalicular membrane in experimental animal models[19–21] and isolated cells.[22]

In non-cholestatic humans given UDCA before undergoing wedge liver biopsy during cholecystectomy, the levels of mRNA encoding numerous transporter proteins, including ABCB11 (BSEP), ABCC2 (MRP2), ABCC3 (MRP3), ABCC4 (MRP4), and ABCB4 (MDR3), remained unaffected, whereas the levels of the Bile Salt Export Pump (BSEP), multidrug resistance-associated protein (MDR3), and multidrug resistance (MRP4) proteins were elevated,[17] suggesting that the regulation of the protein expression by UDCA is post-transcriptional. In the cholestatic rat liver, tauroursodeoxycholic acid (TUDCA) significantly increases the amount of transporter proteins Mrp2 and Bsep, which are present in the hepatocyte canalicular membrane and thereby stimulate biliary excretion of potentially toxic compounds.[19,21] Experimental evidence indicates that TUDCA activates a complex network of signals, which in turn stimulate hepatobiliary vesicular exocytosis and the insertion of transporter proteins into the canalicular membrane of hepatocytes.[10,23] The signals modulated by UDCA include those regulating intracellular Ca^{2+}, protein kinase C (PKC) isoforms, and the cAMP level; these second messengers are thought to modulate the trafficking and insertion of transporters in hepatocytes. TUDCA selectively induces translocation of conventional (c)PKC-α, the calcium-sensitive isoform of PKC, a mediator of regulated exocytosis, to hepatocellular membranes and activates membrane-bound PKC; thus, it is speculated that TUDCA enhances the secretion of bile acids in part through a Ca^{2+}-dependent and PKC-dependent mechanism. However, different PKC isoforms have now been shown to be involved in TUDCA-induced Bsep targeting to the canalicular domain in HepG2 cells transfected with the sodium-dependent bile acid transport protein Ntcp.[22]

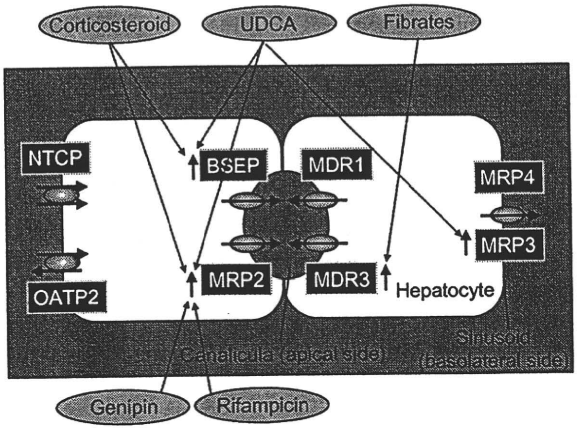

Figure 3 Effect of ursodeoxycholic acid (UDCA) on hepatic transporter expression. UDCA stimulates canalicular Mrp2 and Bsep expression, but also induces basolateral Mrp3, which facilitates alterative efflux of bile acids and other organic anions into the systemic circulation in mice models.[76] In humans, elevated levels of BSEP, MDR3, and MRP4 by UDCA administration was reported.[17] Proposed action point of other drugs used treat cholestatic liver diseases (steroids, fibrates, genipin, and rifampicin) are also demonstrated.

Cyclic AMP is also reported to be a stimulator of the sorting of Mrp2 protein to the apical domain of hepatocytes.[24] In addition, cAMP stimulates the translocation of Ntcp to the plasma membrane through the activation of the protein kinase B signaling pathway.[25] Bile acids, including UDCA, directly inhibit glucagon-induced cAMP formation through a PKC-dependent mechanism,[26] and we have demonstrated that ligation of the bile duct decreases the efficacy of glucagon-stimulated cAMP synthesis by 40–50% without changing its potency in the Syrian hamster.[27] This decreased hormone responsiveness to bile acid is caused, at least

in part, by the desensitization of the glucagon receptor through PKC-induced phosphorylation.[28] We confirmed that the attenuation of glucagon responsiveness by CDCA resulted in the attenuated accumulation of the Mrp2 protein in the canalicular domain of isolated hepatocyte couplets (Ikegami T., 2003, unpublished data), but it is yet to be determined whether this also occurs with UDCA. According to the above reports,[19,21] the alteration in cAMP may not be the only factor controlling the behavior of hepatic transporter proteins; rather, a complex balance between several different signals may decide the final outcome. These alterations in second messengers may be an adaptive mechanism to block the additional uptake of bile acids and to enhance the secretion of potentially toxic intracellular bile acids. However, the exact effects on signaling pathways remain unknown when UDCA is administered to humans.

Cytoprotection and anti-apoptotic action of UDCA

The amphipathicity of bile acids contributes to lipid solubilization, but a higher concentration of bile acids beyond the physiological level often results in disruption of the plasma membrane phospholipid bilayer. In cholestatic diseases, the potent cytotoxic effects of bile acids on hepatocytes and biliary epithelial cells have been discussed. Individual bile acids differ in hydrophobicity.[29] LCA is the most hydrophobic among the major human bile acids, and UDCA is the most hydrophilic. Thus, the composition of bile acids may affect the pathophysiological condition. For instance, an increased concentration of CDCA in the intestinal lumen causes intestinal epithelial cell injury, which often results in the appearance of diarrhea. Partly for this reason, the clinical use of CDCA was abandoned approximately two decades ago. In contrast, UDCA is thought to have a less cytotoxic profile, probably due to its relatively hydrophilic character. Thus, the replacement of hydrophobic bile acids with UDCA may attenuate the damage to hepatocytes and biliary cells, and currently UDCA is administered to patients with chronic viral hepatitis to ameliorate biochemical changes, with the importance of the replacement efficiency of UDCA suggested in this setting. In a multicenter trial in 57 patients with chronic hepatitis due to hepatitis C virus infection (CH-C), Takano *et al.* determined the serum bile acid composition in UDCA-treated patients and found a dose-dependent increase of the UDCA fraction in the serum, a significant correlation between a decrease in serum alanine transferase (ALT), and a decrease in the hydrophobicity index of serum bile acids.[30] This finding was reconfirmed by a recent large-scale, multicenter, double-blinded trial.[31] In this study, the authors concluded that a UDCA dose of 600 mg/day was optimal to decrease serum ALT and aspartate aminotransferase levels in CH-C patients without serious adverse effects, although the long-term effects of UDCA administration on prognosis, hepatocarcinogenesis in particular, remain unclear.

Anti-apoptotic activity of UDCA has been suggested mainly from *in vitro* experiments. In contrast, hydrophobic bile acids have apoptotic effects through the induction of cell death via ligand-independent, death receptor pathways involving the Fas receptor[32,33] and also through classic mitochondrial pathways of apoptosis.[34] Therefore, the final injurious effects of bile acids depend on the balance between insult and protection. There is strong evidence to suggest that the cytoprotective mechanism of UDCA and its conjugates results from the inhibition of apoptosis in hepatic cells by preventing mitochondrial depolarization and reducing the production of reactive oxygen species.[34] UDCA prevents apoptosis by modulating mitochondrial membrane perturbation, opening of the Permeability Transition Pore (PTP), Bax translocation, cytochrome *c* release, and subsequent caspase activation and poly(ADP-ribose) polymerase cleavage.[35] In addition, apoptosis can be inhibited not only by blocking pro-apoptotic pathways, but also by eliciting survival signals through the cAMP, Akt, Nuclear Factor (NF)-κB, mitogen-activated kinase (MAPK), and Phosphoinositide-3 kinase (PI3K)-mediated pathways.[36–38] Tauroursodeoxycholic acid (TUDCA) rapidly inhibits Glycoursodeoxycholic acid (GCDCA)-induced apoptosis in rat hepatocytes independently of the inhibition of caspase 8, the activation of NF-κB, and transcriptional mechanisms.[39] Recently, it has been suggested that the glucocorticoid receptor (GR) plays an important role in the nuclear translocation of UDCA for reducing apoptosis.[40] However, since the anti-apoptotic effects of UDCA have been demonstrated, mainly in experimental models, the relevance of hepatocyte apoptosis in cholestatic liver diseases and the importance of anti-apoptotic mechanisms in the beneficial effects of UDCA remain unclear.

Interestingly, UDCA may also have pro-apoptotic actions, based on the following observations: UDCA potentiates photodamage in leukemia cells;[41] UDCA does not protect against apoptosis induced by hydrophobic bile acids in several colonic cancer cell lines;[42] and UDCA induces apoptosis in hepatocytes when the MAPK and PI3K pathways are inhibited.[43] We have also reported that UDCA potentiates apoptosis induced by a

DNA topoisomerase I inhibitor in colonic and hepatic cancer cell lines.[44] Therefore, UDCA may act differentially on death and survival pathways, depending on the cell type, physiological conditions, and/or stimulus. Thus, further clarification of UDCA action in terms of anti- or pro-apoptotic effects is required to foster a better understanding of the associated mechanisms and to widen the clinical usage of UDCA.

Immunomodulatory effects of UDCA

The GR is considered to be a potential target of UDCA in immune-mediated cholestatic liver diseases, such as PBC. The modulation of cell-mediated immunity by UDCA has been observed,[45] and UDCA has been shown to activate the GR in rat hepatocytes in a ligand-independent fashion, and to suppress interferon-γ-induced Major Histocompatibility Complex (MHC) class II expression in a GR-dependent fashion.[46] In contrast to the binding of dexamethasone (DEX), the binding of UDCA to the rat hepatocyte GR is non-specific.[47] We have also reported that the expression level of type IIA phospholipase A_2 (PLA_2IIA) is downregulated by UDCA via ligand-independent GR activation.[48] Glucocorticoids are known to inhibit PLA_2IIA expression, and there is a putative binding site for the GR located in the −208 to −203 region of the human PLA_2IIA promoter. UDCA may interact with the GR in a different manner to that of the classic receptor–ligand interaction seen with DEX. Our findings that UDCA has an additive inhibitory effect on PLA_2IIA expression above that of DEX and that RU486 (a prototype GR antagonist) can reverse the effect of DEX, but not that of UDCA, are compatible with this hypothesis. Furthermore, the siRNA suppression of the GR levels suggests that the GR is required for the inhibitory action of UDCA.

These findings provide an explanation of the action of UDCA in PBC, which has been considered to be an autoimmune disease. However, further studies are needed to determine if the effects of UDCA on the GR are unique among physiological bile acids and if these effects are secondary to the choleretic effect of UDCA.

EMERGING CONCEPTS FOR UDCA USE

CURRENTLY, THE USE of UDCA in Japan is limited to the treatment of chronic cholestatic diseases (PBC, ICP, and cholestatic drug-induced liver injury), gallstone dissolution or prevention of colic pain, and chronic hepatitis under the regulation of the national health insurance system. However, several other possible applications have been suggested and two of these are described below.

Colon cancer chemoprevention and UDCA

An effect of UDCA on colorectal cancer (CRC) chemoprevention has been shown in preclinical studies.[49–54] UDCA inhibits the proliferation of colon cancer cell lines *in vitro*, and significantly decreases the size and number of colon tumors induced by N-methylnitrosourea[53,54] or azoxymethane[49–51] in rats. In this respect, UDCA has also been shown to be superior to piroxicam, an established chemopreventive agent for colon cancer.[49,50] The chemopreventive mechanism of UDCA in colon cancer has been associated with the reduction of DCA in stools, since DCA has been implicated in the pathogenesis of colorectal cancer through the disruption of the balance between colorectal crypt cell proliferation, differentiation, and apoptosis.[55,56] Secondary bile acids appear to modify intracellular signaling and gene expression,[52,57,58] and DCA in particular appears to stimulate signaling through at least two different pathways that regulate the activity of activator protein-1.[57] Thus, the reduction of fecal DCA in UDCA therapy observed in animal studies may explain the chemopreventive action of UDCA,[59–61] but contradictory results have been obtained in clinical studies. Furthermore, several reports claim that the biological activity of UDCA itself is opposite to that of DCA.[62,63] For instance, UDCA suppresses many of the signaling pathways activated by DCA, such as the MAPK pathway.[62,63] In addition, the modulation by UDCA of changes in PKC isoforms induced by carcinogens[64–66] and changes in arachidonic acid metabolism[51] have been discussed as potential chemopreventive mechanisms. Therefore, UDCA may prevent colon cancer development by the replacement of DCA and through its own molecular actions.

The chemopreventive role of UDCA in CRC has been examined in patients with ulcerative colitis (UC). UDCA treatment has been associated with decreased recurrence rates of colorectal adenomas in patients with a history of PBC after a median intervention period of 45.6 months,[67] and with a lower prevalence of colonic neoplasia in patients with UC and PSC after median treatment periods of 50.4[68] and 42 months,[69] respectively, in a small cohort study. In addition, a recent phase III trial demonstrated a statistically significant 39% reduction in the recurrence of adenomas with high-grade dysplasia ($P = 0.03$), but failed to show a significant difference in total colorectal adenoma

recurrence between UDCA and placebo.[70] The authors concluded that UDCA may work at a later point of colorectal carcinogenesis because of its significant suppressive effect on the recurrence of highly dysplastic adenomas, rather than that of lower-risk adenomas.[70] Collectively, the chemopreventive action of UDCA in colon cancer carcinogenesis has been shown in several clinical studies, but further longer-term studies are required for the clarification of the appropriate population for UDCA supplementation.

Non-alcoholic steatohepatitis and UDCA

Non-alcoholic steatohepatitis (NASH) is a common liver disease that is estimated to affect nearly 1% of the population. The pathophysiology is not clearly understood, but insulin resistance and oxidative stress appear to be involved. Currently, there is no approved therapy for NASH. The treatment of associated conditions, such as obesity, diabetes mellitus, and hyperlipidemia is frequently attempted, but is seldom effective in reversing NASH.[71] UDCA may be a therapeutic option for NASH, because of its multiple hepatoprotective activities in patients with a wide range of chronic liver diseases. Following an open-label pilot study, a large-scale, placebo-controlled trial aimed at determining the efficacy of UDCA for NASH was conducted.[72] However, in this randomized study of 166 patients over 2 years, the administration of UDCA at a dose of 13–15 mg/kg/dL was not better than placebo.[72] A review published by the Cochrane Library in 2007 concluded that there are insufficient data to support or refute the use of UDCA for patients with NASH, since only one[72] of four randomized clinical trials identified was considered to be a low-bias risk trial, and no significant differences were found regarding mortality, improvement of liver function, and the radiological and histological response.

Recent studies have shown that bile acids are also signaling molecules, in addition to their roles in dietary lipid absorption and cholesterol homeostasis. Bile acids inhibit diet-induced obesity and prevent the development of insulin resistance,[73] with several different hypotheses proposed to explain these actions. The direct ability of bile acids to modify the gene expression associated with lipid homeostasis by activating the farnesoid X receptor-á (FXRα), a nuclear hormone receptor, may partly explain these effects.[74] Furthermore, Watanabe *et al.* recently showed that administration of CA to mice increases energy expenditure in brown adipose tissue, thereby preventing obesity and insulin resistance.[75] This effect of bile acids is dependent on the induction of the cAMP-dependent thyroid hormone activating enzyme type 2 iodothyronine deiodinase (D2), since it is lost in mice with targeted disruption of D2. In addition, the effect is independent of FXRα, and instead is mediated by increased cAMP production that stems from the binding of bile acids with TGR5. TGR5 is a novel G-Protein coupled receptor (GPCR) that responds to bile acids by inducing receptor internalization, activation of MAPK pathways, and cAMP production. Taken together, these results suggest that bile acid-controlled signaling pathways are promising targets in the treatment of common metabolic diseases, such as NASH or non-alcoholic fatty liver disease (NAFLD). However, the administration of bile acids, such as CA, that can act as ligands for these receptors may improve lipid catabolism, but also exert a cytotoxic effect. UDCA, a less cytotoxic bile acid, may be useful as an alternative option, but since UDCA has less specific binding to FXRα and TGR5, the modulation of these signaling pathways by UDCA may be relatively weak. The replacement of a more potent bile acid by UDCA may even attenuate the effect of the initial bile acid and result in disease progression. Clinical trials of UDCA in metabolic diseases have not shown a worsened outcome, but currently it appears that a beneficial effect of UDCA in patients with NAFLD or NASH is unlikely.

FUTURE PERSPECTIVES

A VARIETY OF studies have shown beneficial effects of UDCA in hepatobiliary disorders. In clinical practice, UDCA has a defined role in treating patients with cholestatic liver diseases. The safety of the clinical use of UDCA is widely accepted, although the efficacy of UDCA for several clinical conditions is still under debate. Despite frequent novel discoveries of the molecular functions of bile acids, clear evidence for the mechanism of UDCA has yet to emerge. However, further basic research into bile acids may provide this answer and suggest more efficient ways to use this classical compound as a new drug.

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methods

Highly sensitive analysis of sterol profiles in human serum by LC-ESI-MS/MS[S]

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Abstract We have developed a highly sensitive and specific method for the analysis of serum sterol profiles. Sterols in 1 μl of dried serum were derivatized into picolinyl esters (3β-picolinate) and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using the electrospray ionization (ESI) mode. In addition to cholesterol, 19 cholesterol precursors, cholestanol, campesterol, sitosterol, and sitostanol were identified simultaneously. Quantitative analyses for the picolinyl esters of 11 available sterols were performed, and detection limits were found to be less than 1 pg on-column. Reproducibilities and recoveries of 8 noncholesterol sterols were validated according to one-way layout and polynomial equation, respectively. The variances between sample preparations and between measurements by this method were calculated to be 1.6% to 8.2% and 2.5% to 16.5%, respectively. The recovery experiments were performed using 1 μl aliquots of normal human serum spiked with 1 ng to 6 ng of sterols, and recoveries of the sterols ranged from 88.1% to 102.5% with a mean recovery of 98.1%. The present method provides reliable and reproducible results for the identification and quantification of neutral sterols, especially in small volumes of blood samples, which is useful for serological diagnosis of inherited disorders in cholesterol metabolism and for noninvasive evaluation of cholesterol biosynthesis and absorption in humans.— Honda, A., K. Yamashita, H. Miyazaki, M. Shirai, T. Ikegami, G. Xu, M. Numazawa, T. Hara, and Y. Matsuzaki. **Highly sensitive analysis of sterol profiles in human serum by LC-ESI-MS/MS. *J. Lipid Res.* 2008. 49: 2063–2073.**

Supplementary key words cholestanol • cholesterol precursors • congenital birth defect • liquid chromatography-electrospray ionization-tandem mass spectrometry • picolinic acid • plant sterols

Cholesterol is synthesized de novo in virtually all cells of humans and is an essential component of all plasma and intracellular membranes. Recent studies have shown that a number of human malformation syndromes are caused by gene mutations in enzymes for cholesterol biosynthesis after lanosterol (**Fig. 1**). The first malformation syndrome that was confirmed as a defect in cholesterol biosynthetic pathway was the Smith-Lemli-Opitz syndrome (SLOS) due to a deficiency of 3β-hydroxysteroid Δ^7-reductase (1). Afterwards, the cholesterol biosynthetic pathway was investigated in other malformation syndromes and the following deficiencies were discovered, i.e., desmosterolosis (3β-hydroxysteroid Δ^{24}-reductase deficiency) (2), Antley-Bixler syndrome (functional deficiency of lanosterol 14α-demethylase due to cytochrome P450 oxidoreductase gene mutations) (3, 4), hydrops-ectopic calcification-"moth-eaten" (HEM)/Greenberg skeletal dysplasia (3β-hydroxysteroid Δ^{14}-reductase deficiency) (5), congenital hemidysplasia with ichthyosis and limb defects (CHILD) syndrome or NAD(P)H steroid dehydrogenase-like (NSDHL) deficiency (deficiency of 3β-hydroxysteroid dehydrogenase in 4α-methylsterol-4-demethylase complex) (6, 7), CHILD syndrome, X-linked dominant *chondrodysplasia punctata* type 2 (CDPX2) or Conradi-Hünermann-Happle syndrome (3β-hydroxysteroid Δ^8,Δ^7-isomerase deficiency) (8–10), and lathosterolosis (3β-hydroxysteroid 5-desaturase deficiency) (11).

In addition to the deficiency of cholesterol biosynthesis, defects in cholesterol excretion and catabolism are well

Abbreviations: CDPX2, X-linked dominant *chondrodysplasia punctata* type 2; CTX, cerebrotendinous xanthomathosis; ESI, electrospray ionization; GC, gas chromatography; LC-APCI-MS, liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; SLOS, Smith-Lemli-Opitz syndrome; SRM, selected reaction monitoring.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of three tables.

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