Takuya Mohri, Miki Nakajima, Tatsuki Fukami, Masataka Takamiya, Yasuhiro Aoki and Tsuyoshi Yokoi	Human CYP2E1 is regulated by miR-378.	***Biochemical Pharmacology***	79	1045-1052	20010
Masanori Kobayashi, Satorori Higuchi, Katsuhiko Mizuno, Koichi Tsuneyama, Tatsuki Fukami, Miki Nakajima and Tsuyoshi Yok	Interleukin-17 is involved in α-naphthylisothiocyanate-induced liver injury in mice.	***Toxicology***	275	50-57	2010
Katsuhiko Mizuno, Tatsuki Fukami, Yasuyuki Toyoda, Miki Nakajima and Tsuyoshi Yokoi	Terbinafine stimulates the pro-nflammatory responses in human monocytic THP-1 cells throug h an ERK signaling pathwey.	***Life Sciences***	87	537-544	2010
Katsuhiko Mizuno, Yasuyuki Toyoda, Tatsuki Fukami, Miki Nakajima and Tsuyoshi Yokoi	Stimulation of pro-inflammatory responses by mebendazole in human monocytic THP-1 cells t hrough an ERK signaling path wey.	***Archives of Toxicology***	85	199-207	2010
Taiga Maruichi, Tatsuki Fukami, Miki Nakajima and Tsuyoshi Yokoi	Transcriptional regulation of human carboxylesterase 1A1 by nuclear factor–erythroid 2 re lated factor 2 (Nrf2).	***Biochemical Pharmacology***	79	288-295	2010
Ryoichi Fujiwara, Miki Nakajima, Shingo Oda, Hiroyuki Yamanaka, Shin-ichi Ikushiro, Toshiyuki Sakaki, and Tsuyoshi Yokoi	Interactions between human UDP-glucuronosyltransferese (UGT) 2B7 and UGT1A enzymes.	***Journal of Pharmaceutical Sciences***	99	442-454	2010
Shingo Takagi, Miki Nakajima, Katsuhiko Kida, Yu Yamaura, Tatsuki Fukami, and Tsuyoshi Yokoi	MicroRNAs regulate human hepatocytes nuclear factor 4α modulating the expression of metabolic enzymes and cell cycle.	***Journal of Biological Chemistry***	285	4415-4422	2010
Shin-ichi Yokota, Eriko Higashi, Tatsuki Fukami, Tsuyoshi Yokoi and Miki Nakajima.	Human CYP2A6 is regulated by nuclear factor-erythroid 2 related factor 2.	***Biochemical Pharmacology***	81	289-294	2011
Toshihisa Koga, Rhoichi Fujiwara, Miki Nakajima and Tsuyoshi Yokoi.	Toxicological evaluation of acyl glucuronides of NSAIDs using HEK293 cells stably expressing human UGT and human hepatocytes.	***Drug Metabolism and Disposition***	39	54-60	2011

Satonori Higuchi, Masanori Kobayashi, Yukitaka Yoshikawa, Koichi Tsuneyama, Tatsuki Fukami, Miki Nakajima and Tsuyoshi Yokoi.	IL-4 mediated dicloxacillin-induced liver injury in mice.	***Toxicological Letters***	200	139-145	2011
Atsushi Iwamura, Tatsuki Fukami, Hiroko Hosomi, Miki Nakajima and Tsuyoshi Yokoi.	CYP2C9-mediated metabolic activation of losartan detected by a high sensitive cell-based screening assay.	***Drug Metabolism and Disposition***	39	838-846	2011
Yasuyuki Toyoda, Taishi Miyashita, Shinya Endo, Koichi Tsuneyama, Tatsuki Fukami, Miki Nakajima and Tsuyoshi Yokoi.	Estradiol and progesterone modulate halothane-induced liver injury in mice.	***Toxicological Letters***		in press	2011
Hiroko Hosomi, Tatsuki Fukami, Atsushi Iwamura, Miki Nakajima and Tsuyoshi Yokoi.	Development of a highly sensitive cytotoxicity assay system for CYP3A4-mediated metabolic activation.	***Drug Metabolism and Disposition***		in press	2011

III. 研究成果の刊行物・別刷

薬物療法の個人差と薬物代謝酵素の遺伝子多型

Personalized drug therapy and genetic polymorphism of drug metabolizing enzymes

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要　旨

薬の動態（pharmacokinetics）の個人差は極めて大きく、投与された薬の血中濃度に大きな個人差が生じるが、その主たる要因は代謝過程にある。薬物代謝能の個人差は表現型（フェノタイプ）と遺伝子型（ジェノタイプ）に区別して考える。表現型は代謝能で示され、代謝能が欠損または著しく低いヒトをPM（poor metabolizer）と称する。日常的に使用される薬の約8割は、主にチトクロムP450酵素（CYP）によって代謝される。CYPには様々な種類があり、その全てに遺伝子多型が存在する。遺伝子多型の種類と頻度にかなりの人種差が報告されており、各CYPの基質になる薬の整理分類もなされており、臨床における薬物療法への活用が普及しつつある。一方で研究の進展とともに、①代謝能（表現型）に影響を及ぼさない遺伝子変異の報告が増えてきたこと、②表現型に対して、遺伝子多型以外の因子（環境、疾病状態、年齢、嗜好品、飲酒、喫煙、食餌、併用薬、栄養状態など）が複雑に影響を及ぼしていること、③遺伝子型が表現型に及ぼす影響は薬によって大きく異なること等が明らかにされている。こうした点に留意して個別薬物療法（personalized medicine、テーラーメイド医療）を実践する必要がある。

はじめに

日常的に処方される薬による症状の改善の程度は患者によって著しく異なり、疾病によっては患者の半分以上に薬効が認められない場合もめずらしくないと言われている。患者の約10%に何らかの副作用が発現しているというFDAの統計もある。1994年の米国における薬に起因する致死的な副作用の発生率は0.31%（95%信頼区間：0.23-0.41%）と見積もられたという論文が1998年にだされた。これは当時の死因の4～6番目と推定され[1]、薬の副作用による社会的損失は膨大なものであるとされた。時期を同じくして、ヒトゲノムプロジェクトが急速に進捗した結果、21世紀の早い時期に薬の副作用は遺伝子多型を診断することによって予測可能であり、副作用を回避した「個別薬物療法」の時代が訪れると言われ、ファーマコゲノミクス（遺伝薬理学）にかつてない注目が集まった。近年は、遺伝子多型の影響が出来るだけ少ない薬を開発することが指向されている。さらに、臨床試験では被検者の遺伝子多型を考慮した試験が実施されている。我が国においては臨床において、薬物代謝酵素の遺伝子多型の診断は未だに行われていないが、薬物代謝能の個人差に高い関心が集まりつつある。

ファーマコゲノミクスの進歩

薬の体内動態（pharmacokinetics）の個人差は極めて大きい。投与された薬の血中濃度は吸収、分布、代謝、排泄の4つの変動因子によって主に決定されるが、なかでも血中濃度の個人差の主たる要因は代謝過程にあると考えられている。薬物代謝能の個人差は表現型（phenotype）と遺伝子型（genotype）に区別して考える。表現型は代謝能で示され、正常の代謝能を有するヒトをEM（extensive metabolizer）、代謝能が欠損または著しく低いヒトをPM（poor metabolizer）と称する。また著しく高い代謝能を示すUM（ultra rapid metabolizer）や、EMとPMの中間の代謝能を示すIM（intermediate metabolizer）という分類がなされる（図1）。これに対して遺伝子型は、当該DNAの塩基配列の相違を指標として分類され、一般に集団中に野性型（w；wild type）以外の変異遺伝子（alleleまたはアリル）型が1%以上存在する場合を、遺伝子多型があると言う。一般に、変異遺伝子（m；mutated type）をホモで有するヒトはPMの表現型を示す。変異遺伝子をヘテロで有するヒト（wt/m）は、EMとPMの中間であるIMの代謝能を示す場合やEMと変わらない代謝能を示す場合もあり、個々の薬によって異なる。

ファーマコゲノミクスの領域では、薬物代謝酵素の遺伝

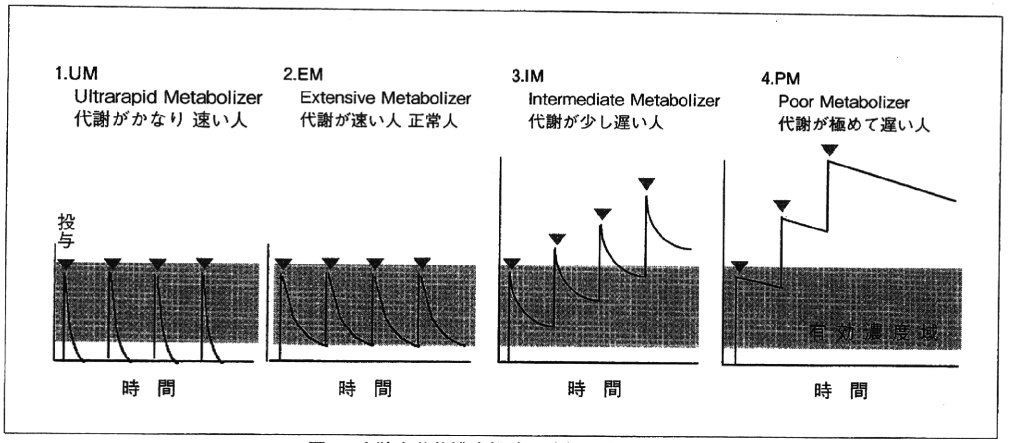

図1 血漿中薬物濃度推移と遺伝子多型

子多型の研究、中でも最も重要な代謝酵素であるチトクロム P450（CYP、Cytochrome P450からCYPと称する）酵素群の研究が進展しており、これまでに膨大な報告がなされている[2]。日常的に使用される薬の約8割はCYPによって代謝される。CYPには様々な種類があり、その全てについて遺伝子多型が存在し、また基質となる薬物の分類整理がほぼ完了している。概略を図2に示す。ヒト肝における各CYP分子種の存在比は、CYP3A4、CYP2C、CYP1A2、CYP2E1、CYP2A6、CYP2D6の順である。これは平均値であり、誘導薬の投与で酵素蛋白の量が増加する分子種としてCYP3A4やCYP1A2がある[3]。また、現在我が国で使われている約3000種類の薬の約半数の代謝にCYP3A4が関与しており、その基質特異性は低い[3]。CYP2D6はヒト肝における含量は3～4%であるが、多くの薬（主に統合失調症治療薬、抗不整脈薬や抗ヒスタミン薬）を特異的に代謝することに注意する必要がある。いずれの薬物代謝酵素にも遺伝子多型が存在し、薬物療法に及ぼす影響の程度は遺伝子変異の種類と薬によって大きく異なる。CYPの詳しい遺伝子多型については国際命名委員会をhttp://www.imm.ki.se/CYPalleles/、個々のCYP分子種の詳しい情報はhttp://www.icgeb.trieste.it/~p450srv/を参照願いたい。

近年、第Ⅱ相の抱合酵素類の遺伝子多型の研究も進捗し、第Ⅰ相酵素とともに、副作用発現との関わりについて明らかにされつつある。さらに、薬物代謝酵素のみならず、トランスポーターやレセプターなどの標的分子の遺伝子多型と薬効および副作用との関連も報告されるようになってきており、表現型には多くの遺伝子が複雑に絡み合っていることが明らかにされつつある。

表1

小児膠原病で使われる治療薬と代謝酵素

1. 非ステロイド系抗炎症薬（NSAID）
   Aspirin（バファリン）CYP2C9で代謝後、UGTで抱合代謝
   Ibuprofen（ブルフェン）CYP2Cで代謝後、UGTで抱合代謝
   Diclofenac（ボルタレン）CYP2C9で代謝後、UGTで抱合代謝
   Naproxen（ナイキサン）CYP2B6、1A2、2Cで代謝後、UGTで抱合代謝
2. 副腎皮質ステロイド薬
   Methylprednisolone（メドロール）CYP3A4を介した相互作用に注意
3. 疾患修飾性抗リウマチ薬（DMARDs）
   MTX（メトトレキセート）ほとんどど代謝されず尿中排泄
   SASP（サラゾピリジン）代謝物5-ASが薬効、TPMTを阻害
   D-penicillamine（メタルカプターゼ）システイン抱合体が尿中排泄
4. 免疫抑制薬
   CyA（ネオーラル）CYP3A4を介した相互作用に注意
   Tacrolimus（プログラフ）CYP3A4を介した相互作用に注意
   Mizoribine（ブレディニン）ほとんど代謝されず尿中排泄
   Cyclophosphamide（エンドキサン）主にCYP2B6で活性化
   Azathioprine（アザニン）6-メルカプトプリンがTPMTによって代謝
   Mycophenolate Mofetil（セルセプト）カルボキシエステラーゼにより活性代謝物であるMPAになった後、UGTで抱合代謝
5. 生物学的製剤
   抗TNFα製剤（レミケード、エンブレル、Humira）
   抗IL-1製剤（Kineret）、抗IL-6製剤（MRA）
   薬物代謝の影響は無い

薬物動態遺伝子の多型と副作用

遺伝子多型と副作用の関係については、CYPの研究領域が最も早くから展開されている。CYPに起因する遺伝子多型と薬物相互作用が問題となり、米国の市場から撤退した薬として、1998年のterfenadine, mibefradil, bromfenac, 1999年のastemizole, 2000年のcisapride, alosetron, 2001年のcerivastatin, 2003年のnefazodoneが知られている[3]。一方、1995年から2000年の18の副作

用調査と論文報告においては、副作用が報告された全ての薬の59%が遺伝子多型を示す酵素によって代謝されるものであったが、売り上げ上位200品目中では、7%であった($P<0.001$)ことから、薬効が優れた薬でも、遺伝子多型を示す酵素で代謝される薬の使用は控えられていることが解る[4]。2004年と2005年の2年間に、米国で患者が救急医療扱いになった薬の副作用事例は、インシュリンに次いでワルファリンが多く(推定43,400件)[5]、これにはCYP2C9とVKORC1(Vitamin K epoxide reductase complex 1)酵素の遺伝子多型が直接関与している。したがって、遺伝子多型に起因する個人差の情報を薬物療法および医薬品開発に反映させることは、副作用を回避し、薬を有効利用するために重要である。

遺伝子多型情報の臨床適用への課題

近年「バイオマーカー」という言葉が多用されている。遺伝子多型は最も有用なgenomic biomarkerの一つということになる。バイオマーカーは、3種類に大別して考えられている[6]。すなわち、確立された方法で測定でき、臨床的にも周知された有用なKnown Valid Biomarker、ある程度確立されているが、臨床的なエビデンスに乏しいProbable Valid Biomarkerと、探索的なExploratory Biomarkerである。こうした分類の考えに基づいて、現在、遺伝子多型と臨床での副作用情報の整理が加速されつつある。

遺伝子多型の情報を個別薬物療法の実践に用いるための条件を以下の5点について考えてみたい。第一に、遺伝子多型情報はPMとEMの表現型(phenotype)を明確に分けることが出来ねばならない。これは当たり前であるが酵素の種類によっては難しい場合がめずらしくない。例えば抗ヒスタミン薬や抗鬱薬を特異的に代謝することで知られる*CYP2D6*には現在までに70種類以上の遺伝子変異が報告されている[7]。しかし、これらの変異の実際に薬の血中濃度への影響について報告がなされている変異は半分にも満たない。さらにその頻度と種類に著しい人種差が存在する。CYP2D6のPMの頻度は欧米人で4～5%であるが、日本人では1%未満である。*CYP2D6*の変異の種類については、欧米人においては、*CYP2D6*3*、**4*と**5*(全欠損型)でPMの約95%を診断でき、*CYP2D6*6*と**7*を加えることによりほぼ全てのPMを診断可能である[8]。一方、日本人ではPMの頻度が低いことに加え、多種類の遺伝子変異が存在するために、臨床研究が進展しておらず、PMの遺伝子診断率は60%程度と考えられる[9]。CYP2D6のみならず、CYP2C9、CYP2C19とUGT1A1の多型の種類の人種差についてFDAも考慮している[6]。日本人においては、CYP2C9とCYP2C19に限っては、数種類の判定でほぼ100%の診断が可能である。

第二に、遺伝子診断が薬物の有効性を高め、副作用を減少させることに役立つことが必須である。飲酒、嗜好品、喫煙、食事、環境因子や併用薬のみならず、年齢、栄養状態や疾病などの様々な遺伝子多型以外の因子が、代謝能に影響する[10]。特に、病態が表現型に及ぼす影響も無視できないと考えられ、疾病時の代謝能と遺伝子型の相関についての情報を蓄積することが求められている。

第三に、遺伝子診断が薬物の選択と投与量の決定に役立つことが必須である。同じ代謝酵素で特異的に代謝される薬物でも、変異の種類によって当該薬の体内動態への影響が大きく異なることが知られている。しかしながら、PMの患者への投与量を明記している薬は無い。この点が大きな問題である。例えば、多動性障害の治療薬であるAtomoxetineは、承認時から、CYP2D6のPMとEMでAUCが約10倍異なることや、t1/2とCmaxが5倍異なることが明記され、パロキセチン(CYP2D6の阻害作用を示す)併用患者やPMに対する注意が喚起されている[11, 12]。しかし、PM患者やパロキセチンなどを併用している患者に対する用量は記載されていないために、臨床現場では直接役には立たない情報である。様々な要因による個体差の影響を考慮すると、用量の明記は難しいと考えられる。しかし、確実な臨床試験データの裏付け無しでは、遺伝子診断でPMと判定しても、実際に用量設定ができない。こうした問題を解決するためには、プロスペクティ

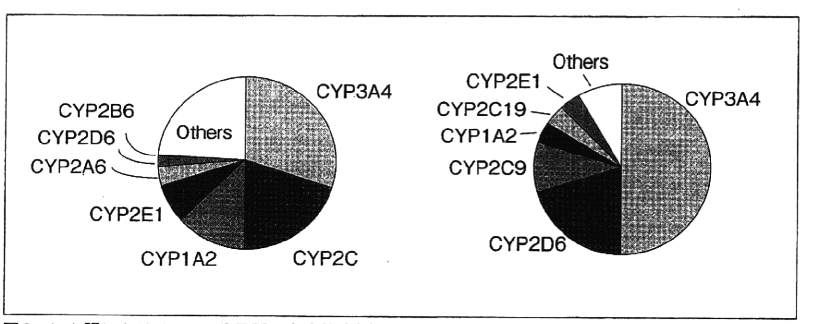

図2　ヒト肝におけるCYP分子種の存在比(左)と、医薬品代謝に関わるヒトCYP分子種の割合(右)

ブな臨床試験が必要であるが、臨床試験には多大な労力と費用を要するために、研究報告がほとんど無いのが現状である。塩酸イリノテカンとUGT(UDP-グルクロン酸転移酵素)1A1の遺伝子多型の関係についても、レトロスペクティブとプロスペクティブな臨床研究が進められている[13-15]。

第四に、遺伝子診断の結果の信頼性が確保され、プライバシーの確保が保証されることが必須である。遺伝子診断は安価で迅速に行うことができるようになった。しかし、多種類の機器や方法によって実施されており、これらの結果の信頼性の確保が重要であり、判定方法、再現性の検証、評価法、提示法、保存法などの統一化が図られる必要があると考えられる[16]。また、遺伝子に含まれる膨大な個人情報はプライバシーの保護の観点から、慎重に取り扱われるべきである。

第五に、遺伝子診断が医療費の節約に結びつくことが必須である。上記の条件を満たし、遺伝子診断を実施することにより、無駄な薬物投与や多剤併用が回避され、さらに、我が国の保険制度においては、最終的には医療費の節約に結びつくことが求められる。こうした理由から、重篤な副作用の発現を確実に予測できる遺伝子診断であっても、変異遺伝子の頻度が極めて低い場合には、多くの患者に不必要な検査の時間と費用の負担を強いることになるので、一般化することは望ましくないと考えられている。

代謝能の個人差と臨床薬物療法

表1に小児膠原病で使用される代表的な薬とその代謝に関する情報をまとめた。最初にCYPで酸化的代謝を受け、その後UGTで抱合代謝を受ける薬が多いが、この場合には、専らCYPの代謝能の個人差の影響を受けると考えられる。特にCYP3A4で代謝される薬が多い為に、CYP3A4で代謝される同効薬や併用薬に充分注意を払う必要がある。複数のCYP分子種で代謝される場合には多型の影響を受け難い。また、治療薬と併用薬が同じCYPで代謝される場合の競合的副作用は、当該薬の代謝酵素に対する親和性の差異で説明できる。臨床で必要な副作用の情報は、添付文書に全て記載されているため、添付文書に常に注意を払う必要がある。

近い将来の薬物代謝酵素の遺伝子診断は、致死的な副作用が予測される場合、または、薬効が得られないと治療が手遅れになる場合など「例えば抗癌剤5-フルオロウラシルやテガフール投与におけるDPYD(ジヒドロピリミジンデヒドロゲナーゼ)の遺伝子多型判定、免疫抑制剤6-メルカプトプリンやアザチオプリン投与におけるTPMT(チオプリンS-メチル転移酵素)の判定や、塩酸イリノテカンの投与におけるUGTの判定等」に限られた範囲で普及することが予測される。UGT1A1分子種の多型については、まもなく我が国でも遺伝子診断が開始されようとしている。

新規のTクラスカルシウムチャンネルアンタゴニストであるミベフラディルは、229名の臨床試験では問題が認められなかったが、2,590名の臨床試験ではCYP3A4が関与すると考えられる相互作用により致死率が上昇するという結果になり発売がキャンセルされた[17]。また、100万件の処方で650例の重篤な肝障害が発症した抗糖尿病薬トログリタゾンについては、複数の動態関連遺伝子の多型が絡み合っていると推定されている[18]。このように1/1,000～1/10,000の患者で発現する重篤な副作用の事前防止に向けて、今後遺伝子多型の情報が活用されていくと思われる。

おわりに

薬物代謝酵素を中心とした遺伝子多型の基礎的研究の成果は、すでに臨床応用可能な段階にある。しかし、個々の薬における表現型と遺伝子型の対応の詳しい情報、特に臨床情報が不足しているために、遺伝子診断の情報を実際の投与量に反映できない場合が多いことが、臨床適用の障害の一つとなっている。今後は、遺伝子の扱いの倫理を考慮しつつ、患者にとって明らかな利益となる遺伝子診断から早急に取り入れる必要があると考えられる。

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Troglitazone

Tsuyoshi Yokoi

Contents

Abstract Troglitazone was the first thiazolidinedione antidiabetic agent approved for clinical use in 1997, but it was withdrawn from the market in 2000 due to serious idiosyncratic hepatotoxicity. Troglitazone contains the structure of a unique chroman ring of vitamin E, and this structure has the potential to undergo metabolic biotransformation to form quinone metabolites, phenoxy radical intermediate, and epoxide species. Although troglitazone has been shown to induce apoptosis in various hepatic and nonhepatic cells, the involvement of reactive metabolites in the troglitazone cytotoxicity is controversial. Numerous toxicological tests, both *in vivo* and *in vitro*, have been used to try to predict the toxicity, but no direct mechanism has been demonstrated that can explain the hapatotoxicity that occurred in some individuals. This chapter summarizes the proposed mechanisms of troglitazone hepatotoxicity based *in vivo* and in vitro studies. Many factors have been proposed to contribute to the mechanism underlying this idiosyncratic toxicity.

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1 Introduction

Thiazolidinediones (Fig. 1) are a class of oral antidiabetic agents, which are a synthetic ligand for the peroxisome proliferator-activated receptor γ (PPARγ) (Lehmann et al. 1995). Troglitazone (Rezulin®, (±)-5-[[4-[(3,4-dihydro-6-hydroxy-2,5,7,8-tretramethyl-2H-1-benzopyran-2-yl)methoxy]phenyl]methyl]-2,4-thiazolidinedione) was the first thiazolidinedione antidiabetic agent approved for clinical use by the US in 1997. Troglitazone lowers blood glucose levels through increased glucose uptake by skeletal muscle, decreased hepatic glucose production, and increased insulin sensitivity of the target tissue in animal models of metabolic impairment (Ciaraldi et al. 1990; Fujiwara et al. 1995). These pharmacological effects are exerted through PPARγ-dependent transcription of genes involved in glucose and lipid metabolism and energy homeostasis (Lehmann et al. 1995; Saltiel and Olefsky 1996; Spiegelman 1998). Based on the pharmacological advantages and the apparent absence of serious toxic effects, troglitazone was thought likely to become a promising treatment for type II diabetes mellitus in patients with insulin resistance.

In the combined North American clinical trials, elevations of serum alanine aminotransferase (ALT) more than three times the upper limit of normal were observed in 48 out of 2,510 patients (1.9%) treated with troglitazone as compared

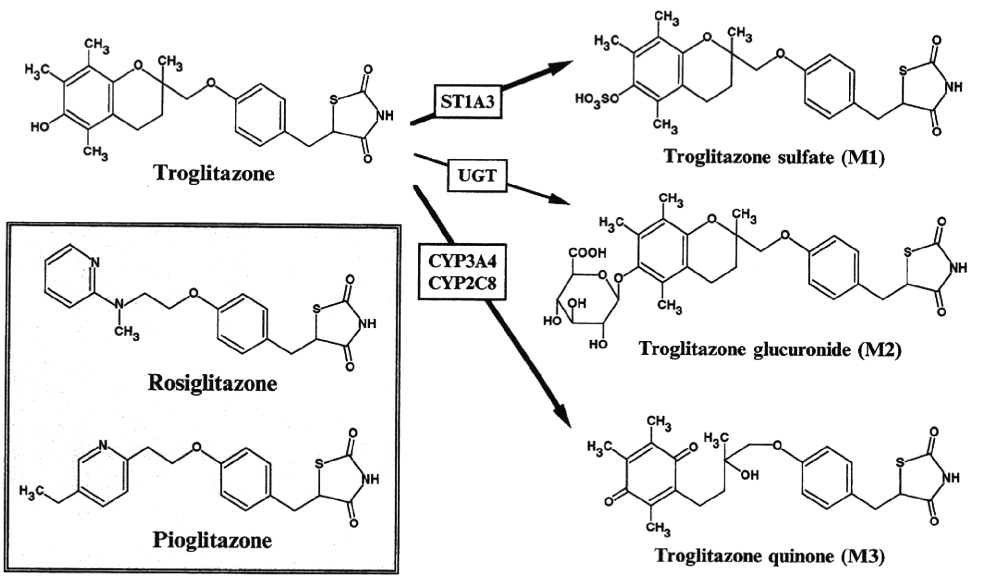

Fig. 1 Structures of thiazolidinediones and pathways of troglitazone metabolism to relatively stable metabolites

to 0.6% in patients who received placebo. Two patients were confirmed to have suffered serious hepatocellular injury from an idiosyncratic drug reaction (Watkins and Whitcomb 1998). Meanwhile, troglitazone was concomitantly reported to be associated with idiosyncratic hepatotoxicity with some patients showing severe or fatal liver damage (Gitlin et al. 1998; Neuschwander-Tetri et al. 1998; Shibuya et al. 1998). Consequently, it was withdrawn from the market in the US and Japan in March 2000. The hepatotoxic effects of troglitazone were not predicted in conventional experimental animals (Watanabe et al. 1999) or in cynomolgus monkeys (300–1,200 mg/kg/day for 52 weeks), a primate model having similar metabolic profiles to humans (Rothwell et al. 2002). Two other thiazolidinediones which are now on the market, rosiglitazone and pioglitazone, were introduced in 1999, and they appear not to exhibit the hepatotoxic effects of troglitazone (Freid et al. 2000; Isley and Oki 2000; Lebovitz et al. 2002), although an association with hepatotoxicity has been reported in very rare instances (Nagasaki et al. 2002). It should also be noted that the clinical dosage regimen for improvement of the fasting glucose level differs among these thiazolidinediones. The recommended dose for troglitazone was 200–600 mg/day, for rosiglitazone 4–8 mg/day, and for pioglitazone 15–45 mg/day (Hanefeld 2001; Loi et al. 1999; PDR 1999, 2005a, b). The Cmax and AUC of troglitazone are 0.90–2.82 mg ml^{-1} and 7.4-22.1 mg-h ml^{-1}, respectively, whereas those of rosiglitazone are 0.076–0.598 mg ml^{-1} and 0.358–2.971 mg-h ml^{-1}, respectively. The plasma elimination half-life and biliary excretion of troglitazone are 16–34 h and 85%, respectively, and those of rosiglitazone are 3–4 h and 23%, respectively. Although the dosage for sufficient pharmacological efficacy could be related to the hepatotoxic potential, the mechanism of troglitazone toxicity is still controversial. Numerous toxicological tests, both *in vivo* and in vitro, have been attempted, but no direct mechanism has been successfully demonstrated that can explain the hepatotoxicity that occurred in some individuals.

Troglitazone represents a model of an idiosyncratic drug reaction that led to withdrawal from the market and to attempts to understand the mechanisms of such adverse drug reactions. This review summarizes the proposed molecular mechanisms of troglitazone hepatotoxicity based on both *in vivo* and in vitro studies. However, so far, there is no direct evidence indicating the precise mechanism of the toxicity. Many factors have been proposed to contribute to this idiosyncratic toxicity.

2 Metabolism of Troglitazone into Stable Metabolites

The bioavailability of troglitazone is 40–50%, which can be affected by food and other factors (Loi et al. 1999). The plasma protein binding is more than 99%, and the distribution into red blood cells is low (Kawai et al. 1997). In humans, there is no evidence that troglitazone accumulates in the liver. However, troglitazone is absorbed in isolated perfused livers and cultured hepatocytes within minutes, even in the presence of albumin or serum (Preininger et al. 1999; Haskins et al. 2001;

Yamamoto et al. 2001). In humans, troglitazone is predominantly metabolized by three pathways: sulfation, glucuronidation, and oxidation, to form a sulfate conjugate (M1), a glucuronide conjugate (M2), and a quinone metabolite (M3), respectively (Fig. 1). The main metabolite, troglitazone sulfate (M1), is catalyzed by phenol sulfotransferase, ST1A3 (Honma et al. 2002), and accounts for about 70% of the metabolites detected in human plasma (Loi et al. 1999), exceeding that of the parent drug. Troglitazone sulfate undergoes enterohepatic circulation after biliary excretion resulting in a long half-life *in vivo* in humans (Kawai et al. 1998), which may be involved in cholestatic liver injury through inhibition of bile acid transport as described below.

A relatively minor metabolite, troglitazone glucuronide (M2), is catalyzed by UGT (Yoshigae et al. 2000). The glucuronidation of troglitazone in human intestine is threefold higher than that in human liver. In the liver, the reaction is likely mediated by UGT1A1, while in the intestine it is mediated by UGT1A8 and UGT1A10 (Watanabe et al. 2002). Furthermore, in enterocytes, it may also be converted to glucuronide by UGTs such as UGT1A8 and UGT1A10.

In human liver, CYP3A4, CYP2C8, and CYP2C19 mainly catalyze troglitazone to a quinone-type metabolite (M3). The chroman ring of vitamin E can be oxidized to a quinone. Kinetic analysis of the troglitazone oxidation (M3 formation) by recombinant P450 enzymes showed that CYP3A4, CYP2C8, and CYP2C19 had relative clearance values of 0.4, 1.6, and 0.9 ml $min^{-1}nmol^{-1}$ P450, respectively (Yamazaki et al. 1999). Considering the relative P450 enzyme contents in human liver, CYP3A4 may be expected to play a major role in the formation of a quinone-type metabolite from troglitazone even at a low concentration. The quinone metabolite M3 is relatively stable and exhibited weaker cytotoxicity than troglitazone (Yamamoto et al. 2001). In addition, troglitazone has been shown to induce CYP3A in human and rat hepatocytes, which stimulates the formation of the quinone metabolites (Ramachandran et al. 1999; Sahi et al. 2000). Therefore, the large interindividual variability of CYP3A4 activities in human liver may be related to the risk of troglitazone-induced hepatotoxicity.

3 Reactive Metabolites and Cytotoxicity

Differing from other thiazolidinediones, troglitazone contains a chroman ring of the vitamin E moiety. This structure accounts for the effective antioxidant property of troglitazone and suggests an advantage in preventing diabetic vascular complications in addition to its hypoglycemic and hypolipidimic effects (Inoue et al. 1997). This structure, however, has the potential to undergo metabolic activation to form the troglitazone quinone metabolite (M3). As mentioned above, although the quinone metabolite M3 is relatively stable, by the action of CYP3As, troglitazone yields several reactive intermediates (Kassahun et al. 2001; Tettey et al. 2001; He et al. 2001) (Fig. 2). The formation of an epoxide of troglitazone quinone was also identified in vitro in humans (Yamamoto et al. 2002) and is likely to be a potent

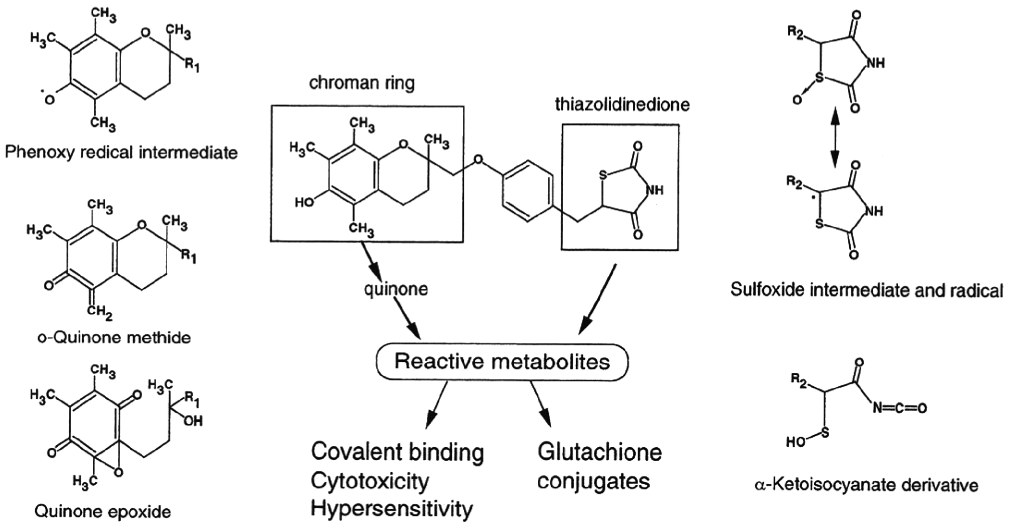

Fig. 2 Reactive metabolites of troglitazone catalyzed by CYP3A potentially leading to toxicity

electrophile. Although the troglitazone quinone does not react directly with GSH, it can be further metabolized to an *o*-quinone methide or undergo ring opening to produce additional highly electrophilic intermediates (Kassahun et al. 2001). Such electrophilic intermediates are toxicologically active, which can result in acute cytotoxicity and immunotoxicity as well as carcinogenesis (Bolton et al. 2000).

Cytotoxicity assays of troglitazone and its metabolites were performed in various types of cells, such as HepG2 cells and hepatocytes from human and experimental animals. The maximum plasma concentrations in patients taking troglitazone at a dose of 600 mg/day only reached about 2.82 μg ml^{-1} or 6.3 μM (Loi et al. 1999). However, a study in rats demonstrated that the concentration of troglitazone in liver tissues was 10- to 12-fold higher than that in the plasma (Sahi et al. 2000). Therefore, the troglitazone levels in human liver might allow the formation of these reactive intermediates, and their accumulation may lead to the hepatotoxicity. In the cytotoxicity assay, the estimated IC50 values of troglitazone and the quinone metabolite, M3, were 34 and 66 μM, respectively, in HepG2 cells incubated in a 5% FBS-containing culture medium (Yamamoto et al. 2001). These reports suggested that the troglitazone levels in human livers could reach such concentrations, which may cause the observed cytotoxicity *in vivo*.

The reactive metabolite(s) covalently binds to cellular macromolecules, but the role of the protein adduct on troglitazone-induced cytotoxicity is still controversial. Using cryopreserved human hepatocytes from 27 individuals, none of the individual phase I or II enzyme activities correlated with the EC50 values of troglitazone cytotoxicity (Hewitt et al. 2002). However, a combination of high CYP3A4 and UGT activities was associated with low toxicity while low CYP3A4 with high ST activity was associated with higher toxicity, which suggested that troglitazone sulfate might act as direct toxicant, and CYP3A4 and UGT were involved in detoxification (Hewitt et al. 2002). On the other hand, chemical inhibitors of drug metabolizing enzymes were employed to elucidate their involvement in the

cytotoxicity to HepG2 cells. Ketoconazole (an inhibitor of CYP3A4), quercetin (an inhibitor of CP2C8), and DCNP (2,4-dichloro-4-nitrophenol, an inhibitor of sulfation) did not successfully attenuate the cytotoxicity in HepG2 cells (Yamamoto et al. 2001). However, inhibition of troglitazone sulfation by DCNP and pentachlorophenol resulted in aggravation of cytotoxicity in human hepatocytes (Kostrubsky et al. 2000), indicating a result opposite to that of Hewitt et al. (2002). The use of cultured cell lines in cytotoxicity assays requires careful interpretation because the activities of drug metabolizing enzymes in such cells are very low. However, Vignati et al. (2005) reported that HepG2 cells, together with microsomes expressing human CYPs or HepG2 cells transfected with CYP3A4, were able to metabolize troglitazone resulting in increased cytotoxicity. Established cell lines expressing the same level of drug metabolizing enzymes as those in human liver would be useful for troglitazone-induced cytotoxicity assays.

4 Biomarkers of Susceptibility to Troglitazone Hepatotoxicity

As mentioned above, troglitazone can undergo metabolic biotransformation by CYP3A4 to form quinone and epoxide metabolites (Izumi et al. 1997a, b; Kawai et al. 1998; Loi et al. 1999; Yamamoto et al. 2002). Quinones are well-established cytotoxic agents and can produce toxicity by redox cycling with molecular oxygen to produce superoxide anion radical and subsequent oxidative stress (Schultz et al. 1996; Bolton et al. 2000). Quinones can also react readily with sulfur nucleophiles such as glutathione (GSH) or cysteine residues on proteins (Bolton et al. 2000). However, little information is available about enzymatic detoxification of these reactive metabolites. The toxic effects of troglitazone have been thought to be mediated by the depletion of GSH, covalent binding to cellular macromolecules, or oxidative stress. In cryopreserved human hepatocytes, large variations in the sensitivity to troglitazone were observed, and sensitive donors were demonstrated to form significantly lower amounts of GSH conjugates and glucuronides than resistant donors (Kostrubsky et al. 2000; Prabhu et al. 2002). It is known that GSH conjugation is catalyzed by the action of glutathione *S*-transferase (GST). A study in rats has shown that GSH adducts of troglitazone are formed and the reaction is enhanced by CYP3As (Tettey et al. 2001). An epoxide of troglitazone quinone catalyzed by CYP3A4 might also be eliminated by GSTs and epoxide hydrolase (Yamamoto et al. 2002). These findings indicate an association between metabolic activation by CYP and detoxification by GSTs. In a key report concerning this aspect, Watanabe et al. (2003) investigated the genetic factors responsible for troglitazone hepatotoxicity *in vivo* in humans. Among 110 patients prescribed troglitazone, 25 had an abnormal increase in ALT or AST levels to at least nine times or five times the upper limit of the normal range, respectively, while 85 control patients showed no significant increase in ALT levels during more than 6 months of treatment. Interestingly, they found that this abnormal elevation of liver enzymes caused by troglitazone treatment was highly associated with the double

null genotype of *GSTM1* and *GSTT1* (odds ratio, 3.692; 95% confidence interval, 1.354–10.066; $P=0.008$) (Watanabe et al. 2003). A similar association study regarding hepatotoxicity observed in patients treated with tacrine, a drug used for Alzheimer's disease, was reported by Simon et al. (2000). Thus, interindividual differences in detoxification ability appears to contribute to the susceptibility and individual risk for troglitazone hepatotoxicity. Taking into consideration the double null genotype of *GSTM1* and *GSTT1* in clinical practice, the risk for hepatotoxicity could theoretically be reduced by half.

Recently, we established a GSH-knockdown rat model for the prediction of human hepatotoxicity (Akai et al. 2007). An adenovirus vector with short hairpin RNA against rat γ-glutamylcysteine synthetase (GCS) heavy chain subunit was constructed and used to knockdown GSH synthesis. This rat model, with an 80% decreased hepatic GSH level, demonstrated a high sensitivity for acetaminophen-induced hepatotoxicity. With the advance of molecular biology, novel animal models will be established and applied to drug development in the near future.

5 Inhibition of Hepatic Drug Transporters by Troglitazone Metabolites

Troglitazone sulfate (M1, the main metabolite) undergoes biliary excretion and accounts for up to 85% of the dose in humans (Loi et al. 1999). In patients with hepatic impairment, troglitazone sulfate was found to accumulate about fourfold in plasma with a threefold increased half-life (Ott et al. 1998; Loi et al. 1999). This metabolite also inhibited the canalicular bile salt export pump (Bsep), organic anion transporting polypeptide (OATP) transporters as well as drug transporters, suggesting it contributes to the hepatotoxicity.

Troglitazone sulfate inhibits the ATP-dependent taurocholate transport mediated by Bsep in isolated canalicular rat liver plasma membrane (IC_{50} 0.4–0.6 μM) about ten times more strongly than the parent compound (IC_{50} 3.9 μM) (Funk et al. 2001a, b). When troglitazone sulfate accumulates in hepatocytes at high concentrations, it may disturb the hepatobiliary export of bile acids by the inhibition of Bsep leading to intrahepatic cholestasis in humans. Evidence of cholestasis has also been described in a patient with troglitazone hepatotoxicity (Gitlin et al. 1998).

Troglitazone sulfate was also reported as a substrate of organic anion transporting polypeptide (OATP) transporters with higher affinity to OATP-C (SLC01B1) than OATP8 (SLC01B3). Estrone-3-sulfate was demonstrated to be a potent inhibitor for OATP-C and OATP8 (Nozawa et al. 2004). Both OATP-C and OATP8 are members of the organic anion transporting polypeptides, which are expressed in the basolateral membrane of hepatocytes (Hagenbuch and Meier 2003; Kreb 2006). They play important roles in the hepatic handling of endogenous compounds and xenobiotics. Some types of genetic polymorphisms with functional alterations of OATP-C have been reported, and such alterations may lead to the accumulation of troglitazone sulfate in the liver, resulting in troglitazone-associated hepatotoxicity

(Kreb 2006, Michalski et al. 2002). Taking such information into account, the failure of hepatic excretion of troglitazone sulfate might lead to hepatotoxicity, although troglitazone sulfate itself is pharmacologically inactive and did not exhibit cytotoxicity in human hepatoma cells (Loi et al. 1999; Yamamoto et al. 2001)

Using knockout rats lacking multidrug resistant associated protein-2 (Mrp2), it has been demonstrated that troglitazone glucuronide is a substrate for Mrp2 (Kostrubsky et al. 2001). Therefore, the troglitazone glucuronide formed in enterocytes might be excreted to the intestinal lumen via transporters such as Mrp2 expressed in the brush border membrane. Then, the glucuronide would again be converted to troglitazone by β-glucuronidase and the troglitazone might be reabsorbed. There has been no reported evidence that M2 is responsible for the hepatotoxic effects of troglitazone.

6 Hypersensitivity Reaction Associated with Troglitazone Hepatotoxicity

Idiosyncratic adverse reactions are difficult to study because of their rare occurrence, dose-independence, and lack of reproducibility in experimental animal models. Many idiosyncratic drug reactions have an immunological (hypersensitivity) basis, whereas some are due to a metabolic abnormality of the host (Pohl et al. 1988; Ju and Uetrecht 2002). Idiosyncratic drug-induced hepatitis has been assumed to be mediated by immunogens formed by covalent interaction of a reactive drug metabolite with cellular macromolecules (Park et al. 1998). The bioactivated immunogens may not only lead to an immune response directed against the haptenic epitope and the neoantigen, but also against autoantigenic determinants, which is characterized by the formation of autoantibodies (Pohl et al. 1988). A number of hepatotoxic drugs have been reported to produce autoantibodies. For example, antiprotein disulfide isomerase, antimicrosomal carboxyesterase, anticalreticulin, anti-ERp72, anti-GRP78, anti-GRP94, and anti-CYP2E1in halothane hepatitis (Bourdi et al. 1996; Gut et al. 1993; Kenna et al. 1993; Pumford et al. 1993), anti-CYP2C9 in tienilic acid-induced hepatitis (Homberg et al. 1984; Robin et al. 1996), anti-CYP1A2 in dihydralazine-induced hepatitis (Bourdi et al. 1990), and anti-CYPs in aromatic anticonvulsant-induced hypersensitivities (Leeder et al. 1992) have been reported. However, it is not fully understood whether the autoantibodies are the causes or consequences of hepatotoxicity. Studies to clarify the possible involvement of autoantibodies in drug-induced hepatitis are limited because the appearance of autoantibodies can usually be seen only in humans. We recently reported that aldolase B, which is an enzyme predominantly localized in the liver and kidney (Penhoet et al. 1966), was detected as an autoantigen that reacted with antibodies in the sera from two patients with type II diabetes mellitus and troglitazone-induced liver dysfunction (Maniratanachote et al. 2005b). The titer of antialdolase B remained high for several weeks after stopping troglitazone administration. This finding supported the idea that troglitazone hepatotoxicity may have an immunological basis.

However, autoantibodies to aldolase B were also detected in the sera of patients with chronic hepatitis as well as liver cirrhosis (Brown et al. 1987; Maniratanachote et al. 2005b). There are several reactive metabolites generated by troglitazone (Fig. 2) (Kassahun et al. 2001; Tettey et al. 2001; Yamamoto et al. 2002). Aldolase B, which is an enzyme predominantly localized in the liver (Penhoet et al. 1966), may be one of the target proteins that interact with those reactive species and trigger the immune response. This study suggested that liver dysfunction might cause the appearance of autoantibodies to aldolase B, which may then aggravate the hepatitis. In addition, the antialdolase B titer might indicate the severity of liver dysfunction. Further studies will be needed to clarify the mechanisms of hypersensitivity reactions.

7 Mechanisms of Troglitazone-Induced Hepatotoxicity

Troglitazone has been shown to induce apoptosis in various hepatic (Bae and Song 2003; Tirmenstein et al. 2002; Yamamoto et al. 2001) and nonhepatic (Shiau et al. 2005) cell types depending on the concentration and duration of exposure. Unlike its pharmacological effects, the toxicity of troglitazone seems to be a PPAR γ-independent mechanism, and the higher affinity PPARγ agonists such as rosiglitazone and pioglitazone possess much lower toxic effects (Lehmann et al. 1995; Shiau et al. 2005). In addition, Shiau et al. (2005) demonstrated that a synthetic counterpart of troglitazone, which lacks PPARγ activation activity, was also able to induce apoptosis in cultured cells. A PPARγ-independent mechanism is also possible in human hepatocytes because the expression of PPARγ in normal human liver cells is very low (Green 1995), and rosiglitazone does not induce apoptosis (Toyoda et al. 2001). Troglitazone was shown to inhibit equally the proliferation of both PPAR$\gamma^{-/-}$ and PPAR$\gamma^{+/+}$ mouse embryonic stem cells (Palakurthi et al. 2001).

As mentioned above, M1, M2, and M3 metabolites are relatively stable, and the quinone metabolite, M3, has been suggested to be associated with troglitazone hepatotoxicity in humans (Neuschwander-Tetri et al. 1998). Although these metabolites showed lower toxic effects compared to the parent compound, troglitazone, when mammalian hepatocytes and hepatoma cell lines were treated directly (Tettey et al. 2001; Tirmenstein et al. 2002; Yamamoto et al. 2001, 2002), the possibility that the metabolites are toxic was not excluded due to the shortage of CYPs and other enzyme activities in the cells. In addition, when exposing the cells to these metabolites, they are unlikely to enter the cells in significant concentrations. On the other hand, it is most likely that troglitazone causes hepatic cell death via apoptosis. Caspase-3 was activated by troglitazone treatment, and pharmacological inhibition of caspase blocked troglitazone-induced cell death (Jung et al. 2007). Apoptosis is a normal physiologic form of cell death and plays a prominent role in liver pathogeneses such as autoimmune liver diseases, viral hepatitis, and drug-induced hepatitis. From this point of view, the cellular, molecular, and *in vivo* responses to troglitazone toxicity will be reviewed in the following sections.

7.1 Mitochondria-Mediated Toxicity

Mitochondria are known to be a source of reactive oxygen species (ROS), suggesting that a direct effect of troglitazone on mitochondrial physiology may play a role in hepatotoxicity (Narayanan et al. 2003). The development of troglitazone-induced toxicity in liver cells could be caused by a reduction of the mitochondrial membrane potential with a concomitant depletion of cellular ATP concentration (Bova et al. 2005; Tirmenstein et al. 2002). Subsequently, it increases the mitochondrial membrane permeability transition and calcium ion (Ca^{2+}) efflux (Masubuchi et al. 2006). The result of these effects on mitochondria is the release of cytochrome c into the cytoplasm and activation of caspases leading to apoptosis (Bova et al. 2005). Using immortalized human hepatocytes, Lim et al. (2008) found that troglitazone rapidly dissipated the mitochondrial inner transmembrane potential, followed by a shift of the redox ratio of mitochondrial thioredoxin-2 (Trx2) toward the oxidized state, and subsequent activation of apoptosis signal-regulating kinase 1 (Ask1). Ong et al. (2007) established heterozygous superoxide dismutase 2 hetero-knockout [Sod2(+/−)] mice as an experimental animal model of silent mitochondrial stress. They found that troglitazone caused liver injury in the high-dose (30 mg/kg/day, i.p.) group, manifested by an approximately twofold increase in serum ALT in Sod2(+/−) but not in wild-type mice. This mouse model could be useful to analyze the dynamics of mitochondrial changes *in vivo* and to investigate the involvement of reactive metabolites in mitochondrial toxicity. Thus, mitochondrial abnormalities could be one of the useful biomarkers of troglitazone-induced idiosyncratic hepatotoxicity.

7.2 Kinase-Mediated Cell Toxicity Pathway

The three well-characterized mammalian mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase (Erk), c-Jun N-terminal kinase (JNK), and p38 are regulated by phosphorylation and play important roles in a variety of cellular processes including growth, differentiation, and apoptosis (Johnson and Lapadat 2002). Erk is generally activated by mitogens, while JNK and p38 are preferentially activated by stress and inflammatory cytokines. The most obvious effect of troglitazone on apoptosis is likely via the promotion of JNK, which in turn activates c-Jun by phosphorylation as well as by activation of p38 (Bae and Song 2003). Gardner et al. (2005) and Jung et al. (2007) reported that calcium/calmodulin-dependent kinase II (CaMKII) is a critical regulator of double-stranded RNA-activated protein kinase (PKR)-dependent p38 and eukaryotic initiation factor 2α (eIF2α) phosphorylation in response to endoplasmic reticulum (ER) calcium depletion by troglitazone. Activation of these kinase-signaling pathways is PPAR γ-independent. In addition, troglitazone also causes the induction of Bax, Bad, the cleavage of Bid, and the release of cytochrome c. Moreover, the mitogen-activated protein kinase (MEK) 1/2-ERK1/2 signaling pathway may be implicated in the

growth inhibitory effect by troglitazone in human cancer cell lines (Motomura et al. 2005; Jung et al. 2007). JNK is characterized as a stress-activated protein kinase based on its activation in response to the inhibition of protein synthesis. These reports suggest that troglitazone induces apoptosis via a caspase-dependent mechanism associated with the downregulation of MEK/ERK and upregulation of p38.

Cyclin-dependent kinases (CDKs) are serine-threonine protein kinases that regulate cell cycle progression. These kinases are activated by various cyclins, inhibited by natural inhibitors such as p21, p27, and p18, and are tightly controlled by transcriptional and posttranscriptional modifications (Sherr and Roberts 1999). Bae et al. (2003) reported that troglitazone-induced cell cycle arrest by this pathway, and apoptosis of hepatoma cell lines ware caused G1 cell cycle arrest through the induction of p53 related proteins and the reduction of cyclin D1, phospho-RB and CDK activities.

7.3 Protein Translation-Associated Toxicity

The endoplasmic reticulum (ER) is a major site of protein synthesis, and its inside or lumen is a major site of protein folding (Gething and Sambrook 1992). In mammalian cells, naturally the rate of protein synthesis is rapidly reduced following the induction of apoptosis. The phosphorylation of eIF2α is important in the regulation of selective translation during ER stress and the unfolded protein response (Holcik and Sonenberg 2005). Troglitazone was shown to promote Ca^{2+} release from the ER leading to PERK and PKR activation, phosphorylation of eIF2α, translation inhibition, and growth arrest (Fan et al. 2004; Gardner et al. 2005).

It is known that the ER is a major cellular storage site of Ca^{2+} in the cell, and that ER chaperones play important roles in Ca^{2+} accumulation and release. Any disturbance in the ER homeostasis causes the release of Ca^{2+}, which in turn blocks ER protein processing. This results in the accumulation of incompletely folded proteins and activates the transcription of ER chaperone genes (Liu et al. 1998; Lodish and Kong 1990). We found that troglitazone treatment of hepatoma cell lines led to overexpression of immunoglobulin heavy chain binding protein (BiP), an abundant chaperone protein in the ER (Maniratanachote et al. 2005a). The important role of this chaperone protein was indicated by the phenotypic change in cell viability when BiP expression was inhibited by small interference RNA (Maniratanachote et al. 2005a). This condition rendered cells more susceptible to the toxic effects of troglitazone. Collectively, it might be postulated that troglitazone acts as a chemical stress signal that causes the release of Ca^{2+} from the ER, and that BiP expression is one of the cellular defense mechanisms of the ER in response to troglitazone-induced toxicity.

Ribosomal protein P0 (P0) was found to be one of the targets of troglitazone cytotoxicity in HepG2 cells (Maniratanachote et al. 2006). P0 is known as a phosphoprotein that functions in the protein translation process (Gonzalo et al. 2001). It was found that, rather than its overexpression, dephosphorylation of P0, which could not be prevented by caspase inhibition, occurred in troglitazone-induced cytotoxicity

(Maniratanachote et al. 2006). Although the dephosphorylation enzyme involved was not identified, a posttranslational modification, dephosphorylation, of P0 was suggested to be associated with the troglitazone-induced toxicity. Proteomics and system biology studies will provide new insights into troglitazone-induced toxicity.

8 Conclusions

Factors affecting the susceptibility to drug-induced hepatic injury include age, sex, co-administered drugs, genetic polymorophism, and enzyme activities catalyzing metabolic activation pathways. Idiosyncratic hepatotoxicity in human is usually unpredictable, pharmacologically independent, very rare, and not reproducible in experimental animal models, which makes it difficult to study (Lee 2003). Troglitazone is known as a typical cause of idiosyncratic hepatotoxicity and has been extensively studied for the past decade. Although a number of toxicological tests, both *in vivo* and in vitro, have been performed, no direct mechanism has been found that can explain why troglitazone hepatotoxicity occurred in only some individuals. We have learned from previous reports that the mechanism of troglitazone hepatotoxicity is PPARγ-independent, that the molecular mechanisms of apoptotic cell death are most likely involved in the hepatotoxicity, and that its idiosyncratic nature may be genetically determined.

Recent findings concerning the miRNA functions in specific tissues has enabled better understanding of the molecular mechanisms of various pathologies and diseases. Among several hundred miRNAs, we first reported the involvement of miRNA on the posttranscriptional regulation of CYPs (Tsuchiya et al. 2006). The decreased expression of miR-27b is one of the causes of the high expression of CYP1B1 protein in humans (Tsuchiya et al. 2006). In addition, we found that miR-148a posttranscriptionally regulated human hepatic pregnane X receptor, resulting in a modulation of the inducible and/or constitutive levels of CYP3A4 in human liver (Takagi et al. 2008). Therefore, studies on miRNAs and their targets could contribute to elucidating the mechanism of troglitazone-induced idiosyncratic hepatotoxicity.

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