仔致死作用は認められないものの、その他の有害作用（変異/骨化遅延、胎仔/新生仔の体重低下、生後の発生指標変化等）が認められる。類薬で、A0 または A1 の条件を満たす。
A2：動物実験において、明らかな胚/胎仔/新生仔の形態異常発生もしくは胚/胎仔/新生仔致死作用が認められている。類薬での動物実験もこれに含める。
AX：類薬を含め、適切な動物実験データがない。

※1：母動物毒性量以上、人常用量 100 倍以上で認められた毒性で、ヒト胎児への影響を直接的に示唆するものではない場合、胎児への影響を過大評価しない。
※2：動物種により結果が異なる場合は、ヒト胎児への影響を直接的に示唆すると判断される場合は A2 とし、ヒト胎児への影響を直接的に示唆するものではない場合 A1 とする。

Q）ＳＥＡ分類に基づくリスクグレーディングは？

A）
以下の通りとする。

ＳＥＡ分類を基にしたリスクグレード
Grade 1；S0 E any A any
Grade 2：S1/X E0 A any
Grade 3：S1/2 E1/2/3 A any、 SX E1/2 A any、SX EX A0/1
Grade 4：S3/X E3 A any、SX E2/X A2
Grade 5：S3/4/X E4 A any、S4 E3 A any

※1：定義上、S2 E0/1/X A any、S3 E0/1/2/X A any、S4 E0/1/2/3/X A any は存在しない。
※2：SX EX AX は「Grade 分類不能」とする。

Q）ＳＥＡ分類に基づく薬剤の総合評価は？

A）
以下の通りとする。

ＳＥＡ分類を基にした総合評価
Ａ：リスクグレードGrade 1のもの
Ｂ：リスクグレードGrade 2のもの
Ｃ：リスクグレードGrade 3のもの
Ｄ：リスクグレードGrade 4のもの
Ｘ：リスクグレードGrade 5のもの
ただし、Ａ－Ｄの薬剤のうち、例えば経口避妊薬など妊娠女性に投与する必要がない薬剤（Not Necessary）については『NN』をつけて『ANN』などと表記する。
また、Ｄ、Ｘの薬剤のうち、より安全な代替薬がなくその女性の生存に必須な薬剤、もしくはその女性の救命に必要な薬剤(Mandatory)については『M』をつけて『DM』、『XM』と表記する。

表　小奇形

＜頭・顔一般＞
1．頭蓋変形
2．三角頭蓋
3．顔面非対称
4．円形顔
5．三角顔
6．扁平な顔
7．老人様顔貌
8．前額突出
9．後頭突出
10．後頭扁平
11．小下顎症
12．下顎後退
13．下顎突出
14．頭皮欠損

＜眼＞
1．眼間開離
2．眼間狭小
3．眼瞼裂斜上
4．眼瞼裂斜下
5．内眼角贅皮
6．眼裂縮小
7．眼瞼下垂
8．眼球陥没
9．眼球突出
10．小眼球(症)
11．青色強膜
12．虹彩欠損(症)
13．斜視
14．角膜混濁
15．白内障

＜耳＞
1．耳介低位
2．耳介変形
3．耳介聳立、ぶらぶら耳
4．大耳(症)
5．小耳(症)
6．耳介前皮膚垂または肉柱
7．耳介前皮膚洞または小窩

＜鼻＞
1．扁平な鼻背
2．高い鼻背
3．小さい鼻
4．くちばし状の鼻
5．球根状の鼻
6．眉間部突出
7．前向きの鼻孔
8．鼻翼低形成

＜口＞
1．小口
2．大口
3．口角の下がった口
4．高口蓋
5．歯列不整
6．二分口蓋垂
7．人中の異常
8．歯牙着色

＜頸＞
1．短頸
2．翼状頸
3．後位毛髪線低位

＜胸腹部＞
1．胸郭変形
2．楯状胸郭
3．漏斗胸
4．鳩胸
5．胸骨短縮
6．乳頭隔離
7．腹直筋離開
8．臍ヘルニア(軽症)
9．鼠径ヘルニア(軽症)

＜外陰部＞
1．尿道下裂
2．停留睾丸
3．小陰茎
4．大陰唇低形成
5．二分陰嚢

＜四肢＞
1．小さな手、足
2．クモ指
3．短指
4．第５指短小、内弯
5．母指低形成
6．幅広い母指
7．母指３指節症
8．屈指
9．指趾の重なり
10．水かき形成

＜皮膚＞
1．母斑
2．血管腫

黒木良和：小奇形のみかたと意義．小児科 Mook 11，1980（一部改変）

２．分類する際の注意点

Q）類薬の定義は？

A）

薬剤の多くで胎児の形態異常発生のメカニズムがわかっていない中で、薬剤による胎児への形態異常発生に関する類薬を体系的に定義するのは困難である。したがって、以下のように類薬を考える。

1）胎児の形態異常発生のメカニズムが既知の薬剤については、臨床用量で同じメカニズムが考えられる薬剤を類薬とする
2）1）以外については、まずは薬理作用の類似性を第一に考える
3）ついで化学構造の類似性を考慮する
4）活性代謝物が類似するものは類薬として取り扱う
5）同じ薬剤であっても体内動態が極端に違う投与ルートの違い（例えば注射剤と貼付剤など著しく胎児の曝露量として違う場合）は類薬として取り扱う

すなわち、薬理学的に類似の作用を持っているか化学構造上類似の構造を持っていて、なおかつ体内動態が類似性を持っている薬剤のうち、胎児の形態異常発生について類似性を持たないと言うことができるもの以外は類薬として取り扱う。

なお、分類の際に類薬で検討した場合には、具体的に何を類薬として検討したか特記事項に記載する。

Q）ＳＥＡ分類とともに記載する内容は？

A）

以下のものを記載する。

『一般名』、『代表的商品名』、『適応症』または『効能又は効果』、『国際誕生年月』、『薬価基準収載年月』、『販売開始年月』、『ＳＥＡ分類を基にしたリスクグレード』、『Ａ－Ｘの総合評価』、『現 FDA 分類』、『オーストラリア分類』、『成育サマリーのサマリー』、『文献』、『特記事項』

また、Ｓ、Ｅ、Ａの各分類結果の理由を簡潔に記載する。

Q）ＳＥＡ分類を調べる時のソースは？

A）

以下の４つを原則とする。

1. Briggs 最新版
2. MEDLINE および医学中央雑誌で検索した文献
3. 動物実験データ
4. 製造承認申請資料

※1：学会要旨は参照しても良いが、分類への利用は慎重に判断する。

Q）ＳＥＡ分類を調べる時の標準文献検索式は？

A）

＜MEDLINE＞

・妊娠中の胎児への影響を調べる際には、『“薬剤一般名” AND (Pregnancy[MH] OR Fetus[MH] OR “Embryonic and Fetal Development”[MH] OR “Abnormalities, Drug-Induced”[MH])』を検索式とする。

・授乳との関係を調べる際には、『“薬剤一般名” AND (lactation[MH] OR Breast

Feeding [MH] OR "Milk, Human" [MH])』を検索式とする。
・Publication Type (PT)の検索としては、"Case Reports"、"Clinical Trial"、"Clinical Trial, PhaseⅣ"、"Controlled Clinical Trial"、"Journal Article"、"Meta-Analysis"、"Multicenter Study"、"Randomized Controlled Trial"、"Review"を用いる。
<医学中央雑誌>
・妊娠中の胎児への影響を調べる際には、『(一般名/TH) and (妊娠/TH or 胎児/TH or 胚と胎児の成長/TH or CK=妊娠, 胎児)』を検索式とする。
・授乳との関係を調べる際には、『(一般名/TH) and (授乳/TH or 母乳/TH)』を検索式とする。

Q) 標準文献検索式で検索した結果、ヒットする論文が極端に多い場合あるいは極端に少ない場合は?
A)
検索式の条件をきつくする、あるいはゆるめても構わないが、その旨特記事項に記載する。

Q) 妊娠時期等との関係については?
A)
S分類およびE分類については、原則として1st trimester (1)、2nd trimester (2)、3rd trimester (3)、授乳期 (L)の4期に分けて、それぞれの分類の記載を試みる。表記は、例えば『S0(1)』、『E1(L)』などとする。
A分類については、例えばラットの器官形成期はラットにとってみれば2nd trimesterとなってしまうが、混同を避けるために、それぞれの動物種の器官形成期のデータはヒトに外挿するかたちで『(1)』と表記する。すなわち表記は、例えば『A2(1)』とする。また、器官形成期前のデータは器官形成期のデータとして取り扱い『(1)』と表記する。一方、器官形成期後のデータは『(2)』もしくは『(3)』と表記する。

Q) ジェネリック薬の取り扱いは?
A)
現時点では、先発品と同じとして扱う。

Q) 妊娠初期流産のデータの取り扱いは?
A)
原則として、ヒトにおける妊娠初期流産に関するデータは分類の際に採用しない。明らかに初期流産を増加させるデータがある場合は、特記事項として付記する。

Q) 代謝産物のデータの取り扱いは?
A)
原則として、代謝産物のデータは分類の際に採用しない。もとの薬剤のデータがない場合で、代謝産物のデータがある場合は、特記事項として付記する。

Q) 児の機能異常の取り扱いは?
A)

児の機能異常データは非可逆性のもののみ分類に採用する。可逆性の児の機能異常データは採用しない。可逆性でも臨床的に十分注意すべき児の機能異常データがある場合のみ、特記事項として付記する。

Q）併用薬による児の形態異常・機能異常等の増強作用の取り扱いは？

A）

分類の際には採用せず、特記事項として付記する。

Q）母体そのものへの悪影響の取り扱いは？

A）

ＳＥＡ分類は本来胎児への影響の分類であるので、妊娠母体そのものへの影響は原則として考慮しない。

Q）ＳＥＡ分類においてぎりぎり基準を満たさない研究の取り扱いは？

A）

例えば当該薬剤服用者 49 例の研究など、ＳＥＡ分類においてぎりぎり基準を満たさない研究については、その研究しか存在しない場合は、分類には採用しないが特記事項として付記する。

Q）S分類において形態異常全体としてのデータと個別の形態異常のデータの取り扱いは？

A）

個別の形態異常については特記事項に記載する。

Q）Ｓ分類において類薬をまとめて行われた研究の取り扱いは？

A）

類薬をまとめて行われた研究で、個別の薬剤の症例数は明示されているものの、結論は全体で示されている場合、その個別の薬剤で統計処理がおこなわれているのでなければ症例シリーズとしてＥ分類に利用する。

Q）Ｓ分類においてS3かS4か迷った場合には？

A）

S3 に分類する。

Q）Ｅ分類における症例シリーズについては？

A）

症例数が一定以上の症例シリーズの研究の結果は、当該薬剤の臨床経験の年数にかかわらず、その結果をもってE1 もしくはE2 に分類してよい。

Q）Ｅ分類において症例報告のみある場合の取り扱いは？

A）

孤発例については、自然発症の危険性との比較結果やその薬剤の作用機序を考慮して、それでも疑わしい場合以外は分類には採用しない。特記事項にも付記しない。なお、この場合は「低頻度」に分類する。

Q）E分類において子宮内胎児発育遅延の取り扱いは？

A）

分類の際には採用しない。それが母体疾患の影響ではなく当該薬剤の影響であることが明らかな場合は特記事項として付記する。

Q）詳細なA分類の検討において最も優先すべきソースは？

A）

上記の原則とするソースで不明な点を調べる際には、PDR (Physicians' Desk Reference)最新版、RXList(http://www.rxlist.com/)、Catalog of Teratogenic Agents (Shepard TH, Lemire RJ) 最新版、Chemically Induced Birth Defects (Schardein JL) 最新版が情報源となるが、企業が持つ医薬品申請時のデータを最も優先する。

Q）A分類において動物実験における薬剤の投与ルートの取り扱いは？

A）

臨床の投与ルートに合致した投与ルートの動物実験データがあれば、それを利用し、ない場合はそれ以外の投与ルートのものから外挿する。

Q）A分類において動物実験における同じ動物種の系統差の取り扱いは？

A）

いちばん鋭敏に出たものをヒトに外挿するのが原則なので、ひとつの系統で異常があれば、たとえ他の系統で異常が認められなくてもそれをA分類に利用する。

Q）SEA分類に基づくリスクグレーディングと妊娠時期等との関係については？

A）

A分類が形態異常発生を中心としたデータに基づくため、リスクグレードに使用するS分類およびE分類については、1st trimesterのものを用いる。それ以外の妊娠時期のS分類およびE分類において、特記すべき事項がある時は『特記事項』欄に記載する。

研究成果の刊行に関する一覧表

書籍：該当なし

雑誌：

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妊娠とくすり
drugs in pregnancy

三橋直樹　順天堂大学教授・産婦人科（静岡病院）

妊娠中の薬剤の使用は胎児の安全を考慮しなければならない．この安全性は経験に基づき科学的に評価されなければならないが，実際は多数ある薬剤ではまだわからない点が多い．動物実験も参考にはなるが，ヒトと実験動物の差があり絶対的なものではない．薬剤の胎児への安全性には薬剤の催奇性の問題と機能障害の問題があり，それぞれ薬剤が投与された妊娠週数に密接に関連している．催奇性では妊娠3週末までは薬剤による流産の可能性はあってもむしろ影響は少なく，最も問題になるのは妊娠4-7週末までである．しかし奇形の種類によっては妊娠15週までは薬剤の危険性を考慮しなければならない．

A. 胎児への安全性を調べるには

薬剤の胎児への安全性についてはさまざまな文書が出ている．それぞれについて検討すると必ずしもリスクの評価が一致していない薬剤があり，臨床上の問題である．

1. 医薬品添付文書　医薬品添付文書には必ず妊娠中あるいは授乳中の使用についての注意が書かれており，最初に参考にするべきである．しかし多くの薬剤では記載が簡単すぎて参考にならない場合も多い．

2. FDA分類，オーストラリア分類　米国など海外での薬剤の安全性の分類で広く参考にされている．表にFDA分類のリスク評価を示した．

3. その他　最近日本でも妊娠中の薬剤についての相談窓口が開設されている（国立成育医療センターなど）．また有名なBriggs（Drugs in Pregnancy and Location）などいくつかの専門書も参考にされている．

表　FDA分類

A：ヒト対照試験で，危険性がみいだされない
ヒトの妊娠初期3か月間の対照試験で，胎児への危険性は証明されず，またその後の妊娠期間でも危険であるという証拠もないもの．

B：ヒトでの危険性の証拠はない
動物生殖試験では胎仔への危険性は否定されているが，ヒト妊婦での対照試験は実施されていないもの．あるいは，動物生殖試験で有害な作用（または出生数の低下）が証明されているが，ヒトでの妊娠期3か月の対照試験では実証されていない，またその後の妊娠期間でも危険であるという証拠はないもの．

C：危険性を否定することができない
動物生殖試験では胎仔に催奇形性，胎仔毒性，その他の有害作用があることが証明されており，ヒトでの対照試験が実施されていないもの．あるいは，ヒト，動物ともに試験は実施されていないもの．ここに分類される薬剤は，潜在的な利益が胎児への潜在的危険性よりも大きい場合にのみ使用すること．

D：危険性を示す確かな証拠がある
ヒトの胎児に明らかに危険であるという証拠があるが，危険であっても，妊婦への使用による利益が容認されるもの（例えば，生命が危険にさらされているとき，または重篤な疾病で安全な薬剤が使用できないとき，あるいは効果がないとき，その薬剤をどうしても使用する必要がある場合）．

X：妊娠中は禁忌
動物またはヒトでの試験で胎児異常が証明されている場合，あるいはヒトでの使用経験上胎児への危険性の証拠がある場合，またはその両方の場合で，この薬剤を妊婦に使用することは，他のどんな利益よりも明らかに危険性の方が大きいもの．ここに分類される薬剤は，妊婦または妊娠する可能性のある婦人には禁忌である．

B. 本邦で問題となることが多い薬剤について

1. 降圧薬　まずチアジド系の利尿薬は妊娠中は原則として使用しない．特に妊娠高血圧症候群（妊娠中毒症）では血液濃縮が起こるためチアジド系利尿薬は使用しない．使用が許されるのは心不全で緊急に利尿が必要な場合のみである．その他の妊娠中の降圧薬で禁忌であるのはアンジオテンシン変換酵素（ACE）阻害薬（カプトリル，レニベースなど）およびアンジオテンシン受容体拮抗薬（ARB，ニューロタン，ブロプレスなど）である．どちらも胎児の腎機能に障害を与え，羊水過少，胎児死亡などを起こす．妊娠前から使用している場合などはほかの薬剤に変更する必要がある．

一方カルシウム拮抗薬は本邦の薬剤添付文書では禁忌となっているが，ほとんど問題を起こすことはない．αあるいはβ遮断薬もほぼ安全である．

2. 甲状腺機能亢進症治療薬　問題のある薬剤はチアマゾール（メルカゾール）である．この薬剤は新生児の頭皮欠損や腸管の異常などの原因となることが知られている．また新生児の甲状腺機能にも影響を与える．したがってチアマゾールは胎児，新生児

に明らかな異常を与える薬剤であるが，一方で，この薬剤を用いなかった場合のリスクの方がはるかに大きいと判断されている．プロピルチオウラシル(PTU) でコントロールできる症例はPTUを使用する．

3. 抗てんかん薬　フェニトイン（アレビアチン），カルバマゼピン（テグレトール），バルプロ酸ナトリウム（デパケン）などの抗てんかん薬は妊娠中に用いると一定の率で胎児，新生児に特有の異常を起こす．胎児異常のリスクは多剤併用で高くなる．しかし妊娠中の発作は母体のみならず胎児にも危険が高く，この群の薬剤は妊娠中でも中止や減量はできない．

4. 糖尿病治療薬　SU剤に代表される内服の糖尿病治療薬は胎盤通過性があり胎児の膵臓機能に影響するだけでなく催奇性も報告されている．一方コントロールの悪い糖尿病は奇形の大きな原因となるので，糖尿病の妊婦は食事療法あるいはインスリンで血糖をコントロールする．

5. その他の禁忌薬　いくつかの薬剤が妊娠中は禁忌とされている．肝炎治療薬のリバビリン（レベトール）は男性配偶者の使用も禁忌である．角化症治療薬であるエトレチナート（チガソン）にも強い催奇性があり妊娠中は禁忌である．同様に大量の合成ビタミンAは妊娠初期には使用しない方が安全である．その他ほとんどの抗悪性腫瘍薬は胎児に障害を与えると考えてよい．しかし，妊娠中にあえてこの種の薬剤の使用を考える場合は，きわめて母体に危険がせまった状態であり，使用にあたっては総合的な判断が必要である．またワルファリンカリウム（ワーファリン）は胎児に対し催奇性があるだけでなく胎児脳出血を起こし，胎内死亡を起こすことがある．その他男性ホルモンは女児に男性化を起こす可能性があるので禁忌である．

C. 妊娠と予防接種

妊娠中最も問題になるのは催奇性のある風疹であるが，麻疹，水痘，おたふくかぜなども妊娠中に罹患すると重症化しやすいのでインフルエンザを含め妊娠前から抗体を持っておいた方が安全である．

1. 風疹，麻疹，水痘，おたふくかぜワクチン　いずれも生ワクチンのため胎児の安全性を考慮し接種前1か月および接種後2か月は避妊することが勧められている．接種時に妊娠していた場合，風疹が特に問題であるが，ワクチン接種が原因となった先天風疹症候群の報告はないといわれている．

2. インフルエンザワクチン　これは不活化ワクチンであり，胎児の安全性の面でほとんど危険性はない．

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Midtrimester termination of pregnancy using gemeprost in combination with laminaria in women who have previously undergone cesarean section

Mana Obata-Yasuoka, Hiromi Hamada, Hideki Watanabe*, Rena Shimura, Maki Toyoda, Hiroya Yagi, Kinuko Takeshima, Kanako Abe, Yoshiko Nakamura, Tsuyoshi Ogura, Yutaka Fujiki and Hiroyuki Yoshikawa

Department of Obstetrics and Gynecology, Institute of Clinical Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan

Abstract

Aim: We aimed to assess the efficacy and safety of midtrimester termination of pregnancy using gemeprost in combination with laminaria in women who had previously undergone cesarean section and in women who had not.

Methods: Between January 1999 and December 2006, we carried out a retrospective study of termination of pregnancy at 12–21 weeks of gestation at the University of Tsukuba Hospital. Termination of pregnancy was carried out by three-step uterine cervical dilation using laminaria followed by vaginal administration of 1 mg gemeprost every 3 h for up to four doses over 24 h.

Results: A total of 173 women underwent midtrimester termination of pregnancy. The women were categorized into two groups: those who had previously undergone cesarean section ($n = 26$) (previous cesarean section group) and those who had not ($n = 147$) (control group). Seven women had undergone cesarean section at least twice. The gemeprost dose administered was 2.8 ± 1.4 mg for the previous cesarean section group and 2.4 ± 1.6 mg for the control group (difference in doses not significant). Although abnormal vaginal bleeding (>500 mL) was more likely to occur in the previous cesarean section group than in the control group (odds ratio, 2.61; 95% confidence interval, 0.63–10.82), none of the woman required blood transfusion. Uterine rupture and failed abortion were not observed.

Conclusion: The efficacy and safety of our laminaria-gemeprost protocol for termination of pregnancy during the midtrimester are similar for women who have previously undergone cesarean section and those who have not.

Key words: gemeprost, laminaria, midtrimester termination of pregnancy, previous cesarean section.

Introduction

Uterine rupture is the most life-threatening complication that can arise during midtrimester termination of pregnancy (TOP), especially in women with uterine scarring. Chapman *et al.* reported that a prior cesarean section (CS) is a risk factor for uterine rupture and blood transfusion in women undergoing

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Reprint request to: Dr Mana Obata-Yasuoka, Department of Obstetrics and Gynecology, Institute of Clinical Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan.
Email: manobata@md.tsukuba.ac.jp
*Present address: Nagato Clinic, Tokyo, Japan

midtrimester TOP.[1] Because the rate of cesarean delivery is increasing, the number of cases of uterine scarring in women undergoing midtrimester TOP will also increase.

At present, midtrimester TOP is more frequently performed by oral and/or vaginal administration of prostaglandin analogues than by surgical methods. The availability and cost-effectiveness of prostaglandins vary among countries. In Japan, oral prostaglandins are not available, and gemeprost (16, 16-dimethyl-*trans* delta-2-prostaglandin E1; Preglandin; Ono Pharmaceutical, Japan), initially designated as ONO 802,[2] is the only licensed vaginal prostaglandin available for midtrimester TOP.

To date, the use of gemeprost for midtrimester TOP in women with uterine scarring has been considered challenging because of the risk of uterine rupture. Cervical ripening before the induction of labor has been practiced widely to decrease the incidence of complications of TOP by both medical and surgical methods. The most common methods for cervical ripening involve laminaria and prostaglandin analogues. TOP by the administration of gemeprost in combination with dilapan[3] or mifepristone[4–7] has also been reported. In a multicenter study conducted in the United Kingdom on TOP using oral mifepristone and vaginal gemeprost, one of 265 patients suffered a uterine scar rupture and a total of 22 patients had previously undergone cesarean section.[4] Laminaria has been compared to mifepristone for cervical ripening in midtrimester TOP; in this study, women with pre-existing uterine scars were excluded.[8] However, the usefulness and safety of laminaria in combination with gemeprost for TOP in women with cesarean scars have not yet been evaluated.

It has recently been reported that TOP by administration of gemeprost alone can be performed as safely in patients with uterine scars as in those without scars.[9–11] Although no difference was observed in the incidence of major complications between patients with and without scars, two cases of uterine rupture were observed among the patients with uterine scars (1.5%).[11]

A safer and more effective regimen with minimal complications should be developed for performing TOP using gemeprost because the use of this prostaglandin is unavoidable in many countries. The objective of the present study was to determine whether gemeprost in combination with laminaria is a safe and effective option for midtrimester TOP in women who have previously undergone cesarean section.

Methods

We studied a consecutive series of cases of termination of singleton pregnancy at 12–21 weeks of gestation at the University of Tsukuba Hospital.

Termination of pregnancy was carried out by uterine cervical dilation with laminaria three times every 12 h for two consecutive days. The number of laminaria was gradually increased from two for the first dilation to 5–7 for the second dilation and 10 for the third dilation. An oral antibiotic (100 mg cefteram pivoxil) was administered after every cervical dilation procedure. On day 3, after the removal of the last laminaria, 1 mg gemeprost was introduced into the posterior fornix of the vagina. Gemeprost was vaginally administered every 3 h for up to four doses on day 3. Patients in whom complete abortion was not achieved by day 3 received further cycles of gemeprost treatment on day 4. Intramuscular administration of ergometrine maleate, ultrasound-guided uterine curettage and intravenous antibiotic injection (1 g cefazolin sodium hydrate) were routinely performed immediately after placental expulsion. The amount of blood loss was determined at 2 h after delivery by weighing the blood absorbed by a disposable paper sheet spread under the parturient woman. We obtained written informed consent from every woman for undergoing TOP according to this protocol.

The main outcome parameters were the total gemeprost dose, total blood loss, and rate of uterine rupture. Minor complications, such as fever and placental retention were closely monitored. Abnormal vaginal bleeding was defined as blood loss exceeding 500 mL during delivery. Failed abortion was defined as the failure to deliver after the completion of three 24-h courses of gemeprost administration, and in such cases, other abortion procedures were considered (oxytocin infusion or surgical uterine evacuation).

Categorical variables were compared using the two-tailed χ^2 test or Fisher's exact test, as appropriate; continuous variables were analyzed using the Mann–Whitney test. A *P*-value less than 0.05 represented statistical significance.

Results

A total of 173 women underwent TOP between January 1999 and December 2006. The women were categorized into two groups: one group comprising women who had previously undergone CS (previous CS group) ($n = 26$; 15.0%) and the other comprising those who

Table 1 Characteristics of patients in the previous caesarean section group and control group

	Previous CS ($n = 26$)	Control ($n = 147$)	
Maternal age (years) (mean ± SD)	33.6 ± 4.1	31.2 ± 5.6	$P < 0.05$
Gestational age (weeks) (mean ± SD)	17.9 ± 2.6	19.1 ± 5	NS
No. previous CS			–
1	19		
2	5		
≥3	2		
No. previous vaginal deliveries (mean ± SD)	0.35 ± 0.68	0.60 ± 0.80	NS
Indication for TOP			NS
Anomalies detected by US (n)	13	63	
Aneuploidy (n)	4	32	
pPROM (severe oligohydramnios) (n)	1	6	
Intrauterine fetal death (n)	4	38	
Maternal disease (n)	4	8	

CS, cesarean section; NS, not significant; pPROM, preterm premature rupture of the membranes; SD, standard deviation; TOP, termination of pregnancy; US, ultrasonography.

Table 2 Outcomes of the termination of pregnancy in the previous caesarean section group and control group

	Previous CS ($n = 26$)	Control ($n = 147$)	
Total dose of gemeprost (mg)			NS
Mean ± SD	2.8 ± 1.4	2.4 ± 1.6	
Range	1–8	1–11	
Blood loss (mL)			NS
Mean ± SD	204 ± 281	167 ± 186	
Range	10–1100	5–1080	
Median	104	110	
IQR	44–192	55–185	

CS, cesarean section; IQR, interquartile range; NS, not significant; SD, standard deviation.

had not (control group) ($n = 147$; 85.0%). In the former group, seven women (26.9%) had undergone at least two CS. No significant difference was observed between the two groups with regard to gestational age or number of previous vaginal deliveries. The indications for TOP were fetal anomaly in 76 women (43.9%), fetal aneuploidy in 36 women (20.8%), severe oligohydramnios resulting from membrane rupture in seven women (4.0%), intrauterine fetal death in 42 women (24.3%), and maternal disease in 12 women (6.9%). The characteristics of the women are compared in Table 1.

The main outcomes of the procedure for the two groups are shown in Table 2. No significant difference was observed in the total gemeprost dose administered between the previous CS group (2.8 ± 1.4 mg) and the control group (2.4 ± 1.6 mg). No significant difference was observed in the mean blood loss between the previous CS group and the control group.

Complications that were encountered are described in Table 3. Abnormal vaginal bleeding (i.e. bleeding exceeding 500 mL during parturition), was observed in three women (11.5%) in the previous CS group and seven women (4.8%) in the control group; (odds ratio, 2.61; 95% confidence interval, 0.63–10.82). Although vaginal bleeding exceeding 1000 mL was observed in one woman in the previous CS group (1100 mL) and in two women in the control group (1080 and 1027 mL), no patient required blood transfusion. Uterine rupture or other complications were not observed in any patient. TOP was successful in all 173 women.

The cumulative abortion rates for the two groups are illustrated in Figure 1. Abortion rate was defined as the percentage of women in whom abortion was achieved for each dose of gemeprost. No significant difference was observed in the abortion rate with 1–4 mg gemeprost between the previous CS group (96.2%) and the control group (91.2%).

Table 3 Complications encountered in patients of the previous cesarean section group and control group

	Previous CS $n = 26$ (%)	Control $n = 26$ (%)	OR (95% CI)
Failed abortion	0 (0)	0 (0)	
Abnormal vaginal bleeding (>500 mL)	3 (11.5)	7 (4.8)	2.61 (0.63–10.82)
Blood transfusion	0 (0)	0 (0)	
Uterine rupture	0 (0)	0 (0)	

CI, confidence interval; CS, cesarean section; OR, odds ratio.

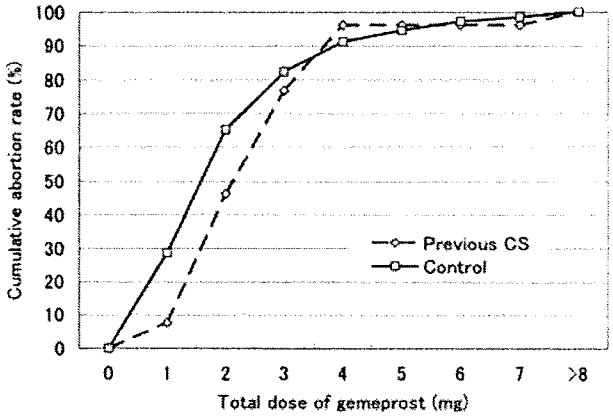

Figure 1 Cumulative abortion rate for the previous caesarean section (CS) group and control group.

Discussion

We used gemeprost (the only licensed vaginal suppository for midtrimester TOP available in Japan) in combination with laminaria for mechanical ripening of the cervix. No significant difference was observed in the total dose of gemeprost administered and the total blood loss between the two groups.

In our study, uterine rupture did not occur in any patient. Le Roux *et al.* reported that after gemeprost administration, uterine rupture occurred in two of five women who had previously undergone CS and in none of the 29 women who had no uterine scarring.[12] Scioscia *et al.* reported the case of a multiparous woman without any uterine scarring who suffered from uterine rupture and underwent total hysterectomy after second trimester TOP with gemeprost.[11] Their protocol involved the administration of gemeprost alone without any pretreatment with a cervical dilator or mifepristone. Although the number of women in our study group was relatively small, on the basis of our results, we can say that pretreatment with laminaria followed by treatment with gemeprost may be safer than treatment with gemeprost alone, even in patients with uterine scars.

Minimal blood loss was observed in most of the women from both groups, and none of the women required blood transfusion. Although the probability of the occurrence of abnormal vaginal bleeding exceeding 500 mL was higher among the women who had previously undergone CS than among those who had not, it did not reach statistical significance (Table 3). This would be partly due to the small sample size of our study. Three cases of vaginal bleeding exceeding 1000 mL were observed, but the appropriate use of uterine contractants after placental expulsion and infusion of Ringer's lactate solution were adequate measures for recovery. Additional hospitalization was not required. The routine use of ergometrine maleate and ultrasound-guided uterine curettage may have contributed to these outcomes. Although the blood loss that occurred with this method of midtrimester TOP is considered to be within the permissible range, even for women who have cesarean scars, we need a study with larger sample size to draw a conclusion.

Some studies have reported on the use of gemeprost with or without laminaria for TOP during the second trimester.[3,6,9,11,12] The mean total dose of gemeprost used in this study (previous CS group, 2.8 ± 1.4 mg; control group; 2.4 ± 1.6 mg) was remarkably lower than the doses used in the previous studies wherein gemeprost was used without laminaria.[9,11] The abortion rate of 91.9% (96.2% in the previous CS group and 91.1% in the control group) with ≤4 mg gemeprost (this presents the total dose when TOP was achieved within 1 day) and the final abortion rate (100% in both groups) were satisfactorily high owing to patient compliance. These results are consistent with those of previous studies wherein gemeprost was used along with other substances for cervical preparations, such as dilapan or mifepristone.[3,6]

In the present study, cervical priming using laminaria was performed before gemeprost was administered. Laminaria absorbs water from proteoglycan complexes in the cervix, leading to dissociation of the complexes, thereby softening and dilating the cervix.[13] Cervical preparation using laminaria is considered very important to prevent cervical lacerations in second-trimester medical abortions, as well as in surgical abortions. In the present study, cervical lacerations as the main cause of excessive bleeding were not observed. In addition to acting as a mechanical modulator, laminaria also acts as a chemical modulator and sensitizes the myometrium to gemeprost.[14] Gemeprost alone can induce cervical ripening as well as labor. Nonetheless, the gemeprost dose can be reduced by exploiting both the mechanical and chemical effects of laminaria. The three-step cervical dilation using laminaria may decrease the total dose of gemeprost required, and TOP using this procedure may be faster and safer than that using gemeprost alone. We consider our regimen is effective and safe for midtrimester TOP in women who have previously undergone CS.

Conclusion

In summary, our findings show that cervical dilation with laminaria followed by vaginal administration of gemeprost can be used effectively and safely for TOP in women who have previously undergone CS as well as for those who have not.

Ethical Approval

The protocol of the present study is in accordance with the Maternal Protection Law (1996) of Japan and the approval of the ethical review committee of the University of Tsukuba Hospital.

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RESEARCH ARTICLE

Gender-related difference in the toxicity of 2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl)benzotriazole in rats: Relationship to the plasma concentration, *in vitro* hepatic metabolism, and effects on hepatic metabolizing enzyme activity

Mutsuko Hirata-Koizumi[1], Kiyomi Matsuno[2], Mitsuhiko Kawabata[2], Kanako Yajima[2], Takashi Matsuyama[2], Akihiko Hirose[1], Eiichi Kamata[1], and Makoto Ema[1]

[1]*Division of Risk Assessment, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan, and* [2]*Shin Nippon Biomedical Laboratories, Ltd. (SNBL), Tokyo, Japan*

Abstract

Previously, we showed that the toxic susceptibility of male rats to an ultraviolet absorber, 2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl)benzotriazole (HDBB), was nearly 25 times higher than that of females. The present study aimed to clarify the mechanism of gender-related differences in HDBB toxicity. Male and female rats were given HDBB by gavage at 0.5, 2.5, or 12.5 mg/kg/day for 28 days, and plasma HDBB levels were measured at various time points by using liquid chromatography–tandem mass spectrometry. HDBB was rapidly absorbed and eliminated from the plasma in both sexes, and no sexual variations were found in the plasma levels. In the plasma, HDBB metabolites were not detected at any dose by the liquid chromatography–photodiode array detector. In an *in vitro* metabolic study using hepatic microsomes from male and female rats, HDBB was slightly metabolized, but no sexual differences were found in the residual HDBB ratio after a 60-minute incubation with an NADPH-generation system. Following 28-day HDBB administration, sexually different changes were found in cytochrome P450–dependent microsomal mixed-function oxidase activities in the liver. In males, 7-ethoxyresorufin *O*-deethylase activity decreased and lauric acid 12-hydroxylase activity increased at all doses. Decreases in aminopyrine *N*-demethylase activity and testosterone 2α- and 16α-hydroxylase activity were also found at 2.5 mg/kg and above in males. In females, the only significant change was increased lauric acid 12-hydroxylase activity at 12.5 mg/kg. These findings indicate that HDBB would have hepatic peroxisome proliferative activity, and the difference in susceptibility of male and female rats to this effect might lead to marked gender-related differences in HDBB toxicity.

Keywords: *Benzotriazole UV absorber; gender-related difference; hepatic metabolizing enzyme activity; in vitro hepatic metabolism; plasma concentration; rat*

Introduction

2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl)benzotriazole (CAS No. 3846-71-7; HDBB) is an ultraviolet (UV) absorber used in plastic resin products, such as building materials and automobile components (METI, 2006). Previously, we showed a marked gender-related difference in the toxicity of HDBB in 28-day and 52-week repeated oral dose toxicity studies using rats (Hirata-Koizumi et al., 2007; 2008a). In the 28-day study, toxic effects were observed mainly in the liver, such as hypertrophy and vacuolar degeneration of hepatocytes, focal necrosis, and bile duct proliferation. HDBB also caused anemia,

Address for Correspondence: Mutsuko Hirata-Koizumi, Division of Risk Assessment, Biological Safety Research Center, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan; Fax: +81-3-3700-1408; E-mail: mkoizumi@nihs.go.jp

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degeneration and hypertrophy of myocardium in the heart, hypertrophy of tubular epithelium in the kidneys, and diffuse follicular cell hyperplasia in the thyroids. Adverse effects were found even at the lowest dose of 0.5 mg/kg in males, but in females, they were detected only at 12.5 mg/kg and above. In the 52-week study, histopathological findings in the liver included precancerous changes (i.e., altered hepatocellular foci). Based on hepatic changes, the no observed adverse effect level (NOAEL) for repeated dose toxicity of HDBB was concluded to be 0.1 mg/kg/day in males and 2.5 mg/kg/day in females. These findings show that male rats have a nearly 25 times higher susceptibility to HDBB toxicity than female rats.

Gender-related differences in the toxic susceptibility of rats have been documented for many other industrial chemicals (Ema et al., 2008; Muraoka and Itoh, 1980), environmental pollutants (Knuckles et al., 2004), insecticides (Agarwal et al., 1982; Carlson and DuBois, 1970), and pharmaceuticals (Coleman et al., 1990; McGovren et al., 1981; Stern et al., 2007; Wang et al., 2001). Various causes of such sexual differences are indicated mainly for toxicokinetic determinants, such as hepatic metabolism (Gad, 2006) and membrane transporter in various organs, including the kidneys and intestine (Morris et al., 2003). For example, Coleman et al. (1990) reported that higher sensitivity of male rats to hematotoxicity of dapsone, which is a major component of the multidrug regimen for the treatment of leprosy, was due to the greater capacity for N-hydroxylation. Another example was an amino-acid antitumor agent, acivicin, of which the LD_{50} was much higher in male mice than in females. McGovren et al. (1981) showed that the plasma half-time was much longer in female mice and speculated that the sexual variation may be related to differences in renal excretion.

The aim of the present study is to clarify the mechanism for gender-related differences in HDBB toxicity. We determined plasma HDBB levels in male and female rats given HDBB, by liquid chromatography–tandem mass spectrometry (LC/MS), and the metabolites in plasma were analyzed by using a liquid chromatography–photodiode array detector. The enzymatic transformation of HDBB was also investigated with hepatic S9 fractions and microsomes prepared from male and female rats, and with the single-enzyme systems, microsomes containing cDNA-expressed individual rat cytochrome P450 (CYP) enzymes. Further, we investigated the effects of HDBB on typical CYP-dependent microsomal mixed-function oxidase (MFO) activities [i.e., aminopyrine *N*-demethylation, 7-ethoxycoumarin *O*-deethylation (ECOD), 7-ethoxyresorufin *O*-deethylation (EROD), testosterone 6β-, 2α- and 16α-hydroxylation, and lauric acid 12-hydroxylation], in the liver, which is the main target of HDBB.

Materials and methods

This study was performed at Drug Safety Research Laboratories (Kagoshima, Japan) and the Pharmacokinetics and Bioanalysis Center (Kainan, Japan) of Shin Nippon Biomedical Laboratories, Ltd. (SNBL) in 2007–2008. The experiment was approved by the Institutional Animal Care and Use Committee of SNBL and was performed in accord with the ethics criteria contained in the bylaws of the Committee.

Materials

HDBB (Lot no. AY11) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The HDBB used in this study was 100% pure and was stored in a light-resistant, tight container at room temperature until use. 2-(3′,5′-di-*tert*-butyl-2′-hydroxyphenyl)-5-chlorobenzotriazole (DBHCB), used as an internal standard for plasma HDBB determination, was also obtained from Tokyo Chemical Industry Co., Ltd. Corn oil, formic acid (special grade), acetonitrile [high-performance liquid chromatography (HPLC) grade] and aminopyrine (for biochemistry) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 7-ethoxycoumarin and 7-ethoxyresorufin were from Sigma-Aldrich Japan K.K. (Tokyo, Japan), and [4-^{14}C]-testosterone and [1-^{14}C]-lauric acid were from GE Healthcare Bio-Sciences KK (Tokyo, Japan). All other reagents and solvents were of the highest quality commercially available.

For *in vitro* metabolism studies, hepatic S9 fractions and microsomes prepared from male and female Sprague-Dawley (SD) rats were purchased from Xenotech LLC (Lenexa, Kansas, USA) and BD Gentest (Woburn, Massachusetts, USA), respectively. The single-enzyme systems, microsomes prepared from baculovirus-infected insect cells expressing CYP1A1, CYP1A2, CYP2A1, CYP2A2, CYP2B1, CYP2C6, CYP2C11, CYP2C12, CYP2C13, CYP2D1, CYP2D2, CYP2E1, CYP3A1, or CYP3A2, were also obtained from BD Gentest.

Animals and housing conditions

Crl:CD(SD) rats (4 weeks old) were purchased from Hino Breeding Center, Charles River Laboratories Japan, Inc. (Yokohama, Japan). After a 7-day acclimation, they were subjected to treatment at 5 weeks of

age. On the day before the first dosing, rats found to be in good health were selected and assigned to three groups of 4 males and 4 females to measure plasma HDBB levels and to four groups of 5 males and 5 females to determine hepatic CYP activity by stratified randomization (MiTOX System, ver. 2.0; Mitsui Zosen Systems Research Inc., Chiba, Japan), according to body weight to minimize bias in body weight among groups.

All animals were maintained in an air-conditioned room at 21.8–22.6°C with a relative humidity of 43–52%, a 12-hour light-dark cycle, and ventilation with 15 air changes/hour. Animals were housed individually in stainless cages suspended over a cage board. A basal diet (CE-2; CLEA Japan, Inc., Tokyo, Japan) and water, which meets the drinking water standard under the Water Works Law of Japan, were provided *ad libitum*.

Measurement of plasma HDBB concentration

Male and female rats (4/sex/group) were given HDBB by gavage once-daily for 28 days. The dosage levels of HDBB were determined to be 0.5, 2.5, or 12.5 mg/kg/day, based on the results of our previous 28-day repeated-dose toxicity study (Hirata-Koizumi et al., 2007). In this previous study, male and female rats were given HDBB by gavage at 0.5, 2.5, 12.5, or 62.5 mg/kg/day, and adverse effects, mainly on the liver, were found at all doses in males and at 12.5 mg/kg and above in females.

Dosing solutions were prepared as a suspension in corn oil. The volume of each dose was adjusted to 10 mL/kg of body weight, based on the latest body weight. The formulations were kept cool in a light-resistant, tight container until dosing and were used within 7 days after preparation. Stability under refrigerated conditions was confirmed up to 7 days in the previous 28-day repeated-dose toxicity study (Hirata-Koizumi et al., 2007).

All males and females were observed twice-daily for clinical signs of toxicity, and body weight was measured on days 1, 7, 14, 21, and 28 of administration. Blood samples (approximately 0.2 mL/animal) were collected from the jugular vein at 1, 2, 5, 8, and 24 hours after the 1st dose, just before the 7th, 14th and 28th doses, and at 1, 2, 5, 8, and 24 hours after the 28th dose. All surviving animals were euthanized by ether anesthesia after the completion of final blood sampling.

The blood samples were centrifuged at 4°C and 1,710 × *g* for 15 minutes to obtain plasma. The plasma (0.05 mL) was mixed with acetonitrile (0.05 mL) and internal standard solution (DBHCB, 0.05 mL) and centrifuged at 12,000 rpm for 5 minutes at 4°C. The supernatant (10 μL) was analyzed by using a CAPCELL PAK C8 DD column [2.0 (inner diameter) × 75 mm, 3 μm; Shiseido Co., Ltd., Tokyo, Japan] on a Shimadzu LC-10A HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a photodiode array detector (SPD-M20A; Shimadzu Corporation) set at 200–400 nm and a triple quadrupole mass spectrometer (API 3000; Applied Biosystems Japan, Tokyo, Japan). The mobile phase consisted of acetonitrile and 0.1% formic acid (75:25, v/v) at a flow rate of 0.2 mL/minute for 15 minutes. Under this condition, the retention time of HDBB was about 9 minutes and the lower limit of qualification was 0.02 μg/mL.

Toxicokinetic parameters of HDBB, maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), and area under the plasma concentration-time curve from time zero to 24 hours (AUC_{0-24h}), were assessed by standard noncompartmental analysis, using WinNonlin version 4.0 (Pharsight Corporation, Mountain View, California, USA).

In vitro *metabolism reaction*

Since the metabolic products of HDBB have not been elucidated, metabolic activity of hepatic S-9 fractions and microsomes from male and female rats was determined by measuring the disappearance rate of HDBB after incubation. HDBB was dissolved in acetonitrile at 4.5 mmol/L, and 0.005 mL of the HDBB solution was incubated with 0.05 mL of S-9 fractions or microsomes (20 mg/mL) in 0.1 mol/L of phosphate buffer (pH 7.4) containing 0.05 mmol/L of EDTA. Final HDBB concentration in the incubation mixtures was 45 μmol/L. The incubation was carried out at 37°C in air. After a 5-minute preincubation, the reaction was initiated by adding the NADPH-generating system (15.5 mmol/L $NADP^+$, 33 mmol/L glucose-6-phosphate, 4 U/mL glucose-6-phosphate dehydrogenase, and 33 mmol/L $MgCl_2$), and incubated for 60 minutes. The reaction was terminated by the addition of 1 mL of ice-cold acetonitrile, and the solution was centrifuged for 15 minutes at 10,000 × *g* and 4°C. The supernatant (0.05 mL) was eluted by using the above-mentioned HPLC system, and the elution was monitored at 346 nm with a Shimadzu SPD-10A or 20A UV detector (Shimadzu Corporation). All experiments were performed in duplicate. The residual HDBB ratio was calculated by dividing the peak area of HDBB after a 60-minute incubation with that of the control, in which the incubation system was inactivated by the addition of 1 mL of acetonitrile prior to incubation ($n = 1$).

To examine the role of individual CYP isoforms involved in the metabolism of HDBB, each of the

recombinant CYPs (200 pmol of CYP/mL) was incubated with HDBB, using the same method as mentioned above, except that potassium phosphate buffer was used instead of phosphate buffer. Microsomes from insect cells infected with wild-type baculovirus (BD Gentest), which contains negligible amounts of CYP, served as controls.

Effect of HDBB on Hepatic CYP Activity

HDBB was administered by gavage to male and female rats (5/sex/group) at 0.5, 2.5, or 12.5 mg/kg/day for 28 days. Control groups (5 males and 5 females) received the vehicle only. Preparation of the dosing solutions, observation of the clinical signs of toxicity and measurement of body weight, was performed in the same way as the above-mentioned study for determining plasma HDBB levels. The day after the last administration, the animals were euthanized by exsanguination under deep anesthesia by the intraperitoneal (i.p.) injection of pentobarbital sodium. The surface of the body, organs, and tissues of the entire body were observed grossly. The liver was then collected and weighed. After perfusion to remove blood, the right lobe was homogenized in a 9-fold volume of ice-cold Tris buffer (50 mmol/L Tris-hydrochloric acid buffer containing 0.25 mol/L sucrose; pH 7.4) and centrifuged at 9,000 × *g* for 30 minutes. The supernatant was centrifuged at 105,000 × *g* for 60 minutes, the pellet was suspended in Tris buffer, and centrifugation was repeated. These preparations were performed at 4°C. The resulting pellet was suspended in Tris buffer in an amount equal to the liver weight and used as hepatic microsomes.

The concentration of hepatic microsomal protein was determined by using the Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Inc., Hercules, California, USA) with bovine serum albumin (BSA) as a standard. The total CYP content was measured by the CO difference spectrum method (Omura and Sato, 1964). Seven types of MFO activities (i.e., aminopyrine *N*-demethylation, ECOD, EROD, testosterone 6β-, 2α- and 16α-hydroxylation, and lauric acid 12-hydroxylation activity) in hepatic microsomes were determined by standard procedures. Briefly, aminopyrine *N*-demethylase activity was assayed by determining the formation of monomethylol dimethylhydantoin from aminopyrine spectrophotometrically. ECOD and EROD activities were measured as the rate of conversion of 7-ethoxycoumarine to 7-hydroxycoumarin, and of 7-ethoxyresorufin to resorufin, respectively, using a spectrophotofluorometer. Testosterone 6β-, 2α-, and 16α-hydroxylase activities and lauric acid 12-hydroxylase activity were assayed by using ^{14}C-labeled substrates, and the respective activities were determined by quantifying the formed amount of 6β-, 2α-, and 16α-hydroxytestosterone and 12-hydroxylauric acid by radio-HPLC. Each type of MFO activity was assayed by using NADPH as the sole electron source.

Data analysis

Body weight and absolute and relative liver weight were analyzed by Bartlett's test for homogeneity of variance ($P < 0.05$). When homogeneity was recognized, Dunnett's test was applied to compare the mean value in the control group with that in each test article group ($P < 0.01$ or 0.05). If not homogenous, the data were rank-converted and a Dunnett-type test was applied ($P < 0.01$ or 0.05).

For metabolic enzyme activity, Bartlett's test was similarly performed ($P < 0.05$). When the variance was homogenous, Williams' test, assuming a dose-related trend, was applied ($P < 0.05$). If significant differences were not detected by the Williams' test, the data were further analyzed by Dunnett's test to compare between control and individual treatment groups ($P < 0.05$). When the variances were heterogeneous, the Shirley-Williams' test, assuming a dose-related trend, was performed ($P < 0.05$). If no significant differences were found, Steel's test was applied ($P < 0.05$).

Results

HDBB administration for 28 days did not induce any clinical signs or affect the body weight, except for significantly increased body weight on day 14 of administration in males in the 0.5 mg/kg group. At the completion of 28-day administration, the liver was grossly enlarged in 5/5 males and 1/5 females at 12.5 mg/kg, 5/5 males at 2.5 mg/kg, and 1/5 males at 0.5 mg/kg. In the liver, white focus was found in 4/5 males and 2/5 females at 12.5 mg/kg and in 4/5 males at 2.5 mg/kg. Absolute and relative liver weight was significantly increased at 2.5 mg/kg and above in males and at 12.5 mg/kg in females, as shown in Figure 1. There was also an increase in absolute and relative liver weight at 0.5 mg/kg in males, but no statistically significant difference was found from the control.

Plasma HDBB concentration

The time course for levels of HDBB in male and female plasma after the first intragastric administration is shown in Figure 2A. HDBB was rapidly absorbed and

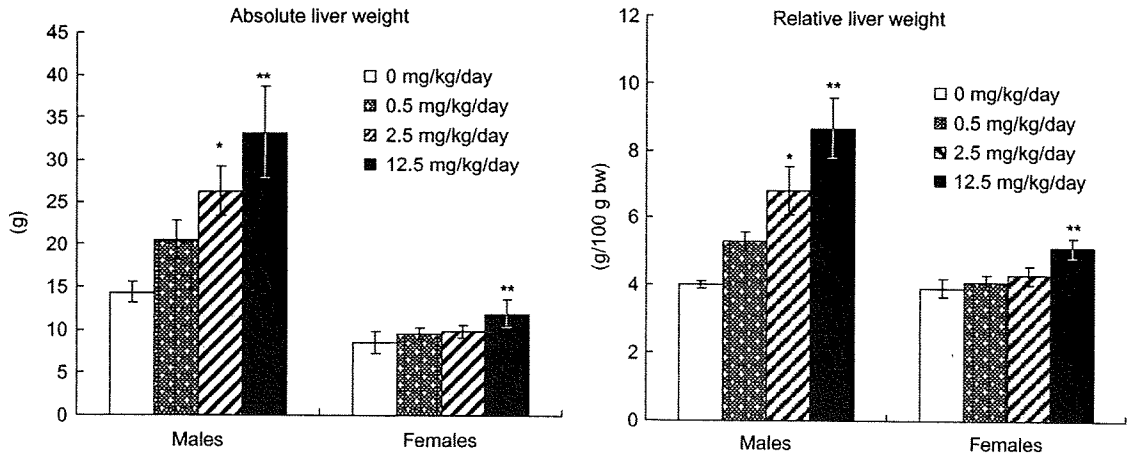

Figure 1. Absolute and relative liver weight of male and female rats given HDBB by gavage for 28 days. Data are expressed as the mean ± standard deviation (SD). *Significantly different from the control, $P < 0.05$; **Significantly different from the control, $P < 0.01$.

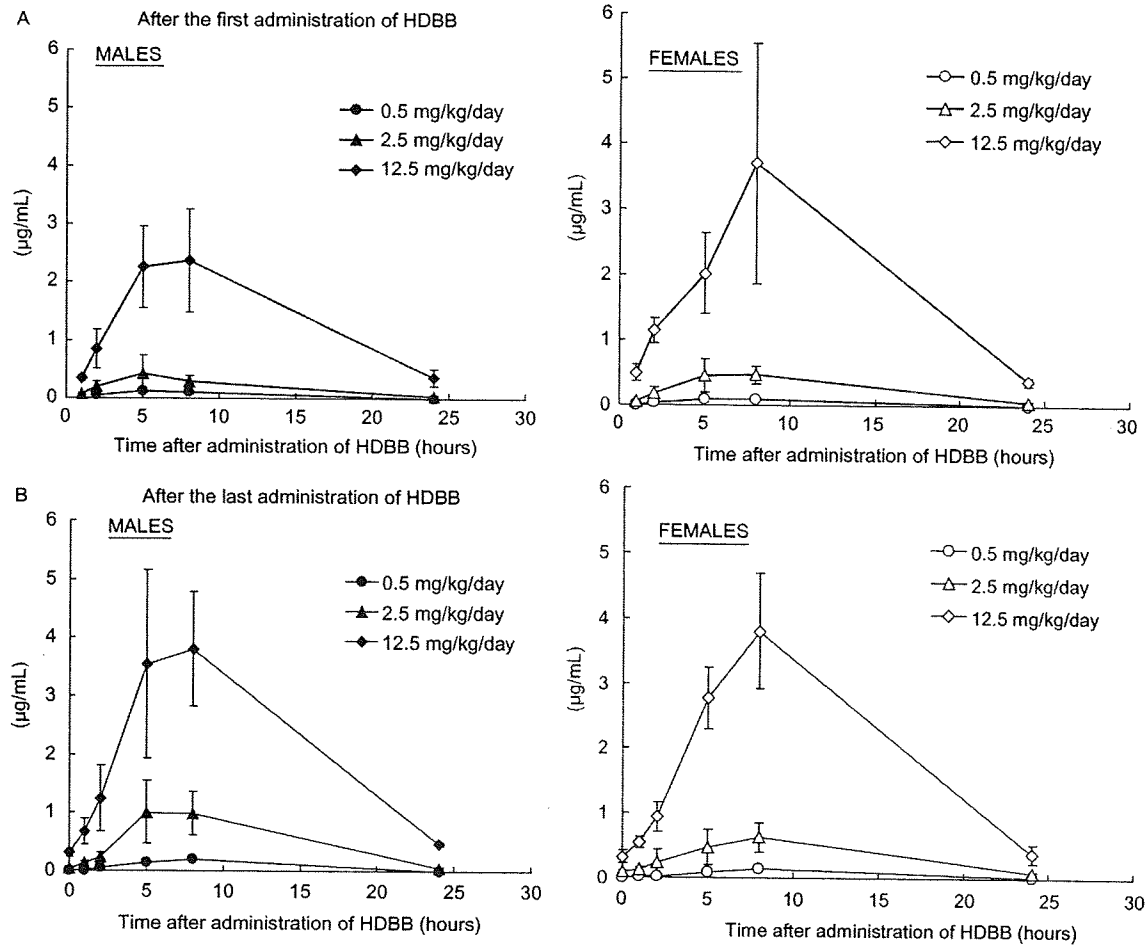

Figure 2. Plasma HDBB concentrations against time after the administration of HDBB to male and female rats. Data are expressed as the mean ± SD.

eliminated from the plasma in both sexes. No clear gender-related differences were found in the plasma profiles of HDBB at any doses. After 28-day repeated-dose administration, similar plasma HDBB profiles were observed, and there were no gender-related differences in the profiles (Figure 2B). In all dose groups, HDBB metabolites were not detected in the plasma at any sampling times in either sex.

The calculated values of C_{max}, T_{max}, and AUC_{0-24h} for HDBB in plasma are given in Table 1. The data showed that AUC_{0-24h} as well as C_{max} values increased in rat plasma with the higher HDBB dose. Comparison of data for males and females indicated no gender-related differences.

Table 1. Toxicokinetic parameters of HDBB.

Doses	Sexes	C_{max} (μg/mL)	T_{max} (h)	AUC_{0-24h} (μg·h/mL)
After the first administration of HDBB				
0.5 mg/kg/day	Males	0.145±0.031	5.75±1.50	1.59±0.32
	Females	0.116±0.036	5.75±1.50	1.25±0.10
2.5 mg/kg/day	Males	0.484±0.276	5.75±1.50	4.99±1.45
	Females	0.573±0.165	7.25±1.50	6.65±1.61
12.5 mg/kg/day	Males	2.85±0.64	6.50±1.73	34.4±7.1
	Females	3.84±1.71	7.25±1.50	47.1±15.7
After the last administration of HDBB				
0.5 mg/kg/day	Males	0.214±0.054	6.50±1.73	2.49±0.62
	Females	0.154±0.009	8.00±0.00	1.98±0.15
2.5 mg/kg/day	Males	1.14±0.42	5.75±1.50	13.6±5.0
	Females	0.636±0.221	7.25±1.50	8.89±3.25
12.5 mg/kg/day	Males	4.27±0.96	5.75±1.50	54.0±11.4
	Females	3.80±0.89	8.00±0.00	50.1±9.8

Values are expressed as the mean ± SD.

In vitro metabolism reaction

After a 60-minute incubation using the liver S-9 fraction prepared from male or female rats with the NADPH-generating system, the concentration of HDBB in the incubation mixture was hardly changed, as shown in Figure 3. The mean residual ratio of HDBB was 98.1% with male rat hepatic microsomes and 91.4% with female rat hepatic microsomes. On the other hand, when incubated with male and female hepatic microsomes, HDBB concentration in the incubation mixture decreased to 73.4 and 76.1% of the control, respectively. In either male or female microsomes, another peak was found around a retention time of 1-2 minutes.

Figure 4 represents residual ratios of HDBB after a 60-minute incubation with microsomes containing cDNA-expressed individual rat CYP enzymes in the presence of the NADPH generating system. Among the 14 types of CYP isoforms tested here, CYP1A1 exhibited the greatest metabolic activity of HDBB (mean residual HDBB ratio: 61.8%). CYP1A2, 2A2, 2B1, 2C6, 2C11, and 2D2 also metabolized 10-20% of HDBB. Other CYP isoforms, CYP2A1, 2C12, 2C13, 2D1, 2E1, 3A1, and 3A2, showed no significant metabolism of the chemical (mean residual ratio of HDBB: >95%). After incubation with CYP1A1, 1A2, 2A2, 2C6, 2C11, 2D2, 3A1, or 3A2, some peaks other than HDBB were detected.

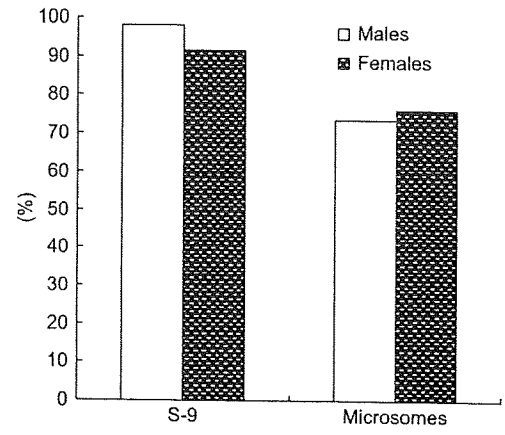

Figure 3. Residual ratios of HDBB after incubation with male and female rat liver S-9 and microsomes in the presence of a NADPH-generating system. Data are expressed as the mean values of two determinations. Residual HDBB ratio was calculated by dividing the peak area of HDBB after a 60-minute incubation by that of the control, in which the incubation system was inactivated by the addition of 1 mL of acetonitrile prior to incubation.

Effect of HDBB on hepatic CYP activity (Table 2)

While microsomal protein content showed no significant differences between HDBB-treated and control groups, the total CYP content was significantly increased in males of the 2.5 and 12.5 mg/kg groups. In these groups, aminopyrine *N*-demethylase activity, and testosterone 2α- and 16α-hydroxylase activity, decreased significantly. EROD activity showed a significant decrease in males in all HDBB-treated groups. These changes were not detected in females. Lauric acid 12-hydroxylase activity was significantly increased at 0.5 mg/kg and above in males and at 12.5 mg/kg in females. No significant changes were found in ECOD activity or testosterone 6β-hydroxylase activity in either sex.

Discussion

The current study was conducted to clarify the mechanism of marked gender-related differences in HDBB