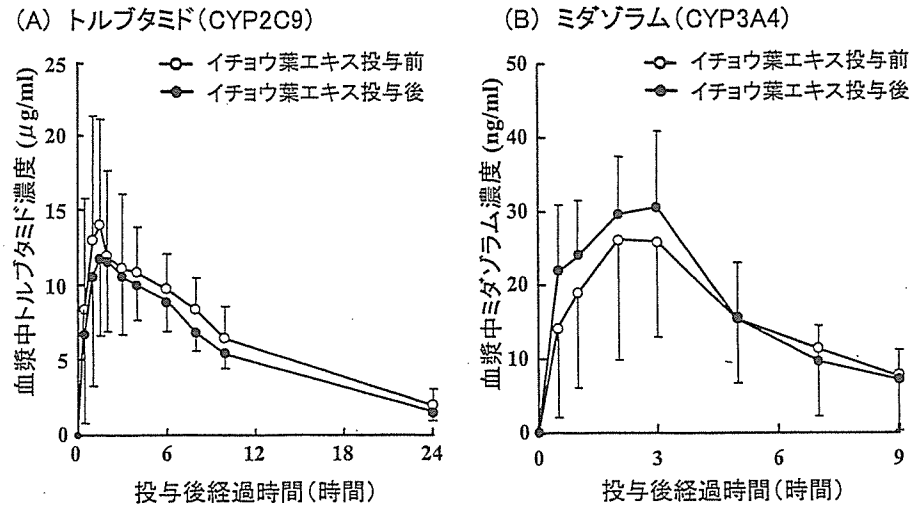

図4　健常成人におけるイチョウ葉エキス28日間投与後のトルブタミドおよびミダゾラムの血漿中濃度推移

時間曲線下面積（AUC）はいずれも投与前と比べ17％有意に減少し，ミダゾラム経口投与後AUCはイチョウ葉エキス投与前に比べ25％有意に増加した（図4）。さらにトルブタミドによる血糖低下作用はイチョウ葉エキス投与により減少する傾向が認められた。これらの結果はイチョウ葉エキスがCYP2C9に対しては誘導作用を，CYP3A4に対しては阻害作用を有する可能性を示すものである。CYP2C9はスルホニルウレア薬，ワーファリン，アンジオテンシンⅡ受容体拮抗薬などの代謝に関与し，CYP3A4はベンゾジアゼピン系薬物，カルシウム拮抗薬など数多くの薬物を代謝することが知られており，これらの代謝酵素の基質となる薬物とイチョウ葉エキスの併用は薬物動態上の相互作用を惹起し，薬物作用を変化させる可能性が示唆される。

我々の結果は，イチョウ葉エキスがCYP3A4の基質となるニフェジピンの血中濃度を53％上昇させたとするSmith氏らの報告[10]とも一致するが，一方，Gurleyらは12名の健常者にイチョウ葉エキスを4週間投与し，CYP1A2，CYP2D6，CYP2E1，CYP3A4の活性に及ぼす影響を検討し，いずれのCYPに対しても有意な変化を及ぼさない事を報告している[11]。このようにイチョウ葉エキスによるCYPへの影響はいまだ不明な点が残されている。

3.3　薬力学的相互作用

イチョウ葉エキス中に含有されるフラボノイド類やギンコライドは，末梢循環改善作用，冠動脈拡張作用や血小板活性化因子抑制作用を有する事が知られている。イチョウ葉エキスは，酸化ストレス下での血小板凝集を特異的に抑制し，また，その併用によりチクロピジンの作用を増強することが報告されている。健常な33歳女性における両側性くも膜下出血[12]や70歳のアスピリン併用者での眼底出血[13]，78歳のワーファリン併用者での脳出血[14]とイチョウ葉エキス摂取との関連を示唆する症例が報告されており，抗血小板薬や抗凝固薬との併用時には，出血傾向を上昇させる可能性がある。一方，ランダム化プラセボ対照二重盲検比較試験では，イチョウ葉エキスの併用が至適効果を保つワーファリンの維持量を変化させなかった事が報告されている[15]。

近年の代替医療やサプリメントへの関心の高まりから，医薬品間の相互作用だけではなく，健康食品やサプリメントと医薬品との相互作用にも注意する必要がある。しかし，サプリメントと医薬品との臨床的な相互作用に関するエビデンスは決して十分とは言えず，イチョウ葉エキスに関しても，その有害事象は症例報告によるものが多く，真にイチョウ葉エキスの併用に関連して生

じたものであるのかを評価することは困難である。今後，イチョウ葉エキスのCYPに対する影響や，抗血小板薬・抗凝固薬との相互作用などについて，そのメカニズムを含めたより詳細な科学的検証が望まれる。

4 おわりに

本稿では，ノコギリヤシ果実抽出液とイチョウ葉エキスについて有効性や医薬品との相互作用に関する知見を，我々の検討を中心に紹介した。メディカルハーブを含めいわゆる健康食品の摂取は，医薬品とは異なり，通常，一般消費者の判断によって行われる。さらに健康食品と医薬品を併用する場合ですら，その摂取は患者自身の判断により行われ，医師や薬剤師が医薬品との併用を知らないことが多いと指摘されている。独立行政法人国立健康・栄養研究所ではウェブサイト上において「健康食品の有効性・安全性情報」[16]を公開し，健康食品，サプリメントといった補完代替医療の有効性及び安全性に関する情報を提供している。このような一般消費者と医療従事者に対する情報提供は，今後ますます重要になるであろう。

代替医療や健康食品の関心とその有用性への期待がますます高まっている今日，いわゆる健康食品の有効性に対する科学的根拠を与えるとともに，医薬品との相互作用を含め有害作用に対する評価は，健康食品の適切な利用と危害防止に大きく寄与すると期待される。

参考文献

1) S. Gutow, *J. Urol.*, 169, 16A (2003)

2) E. Koch, *Planta Med.*, 67, 489-500 (2001)

3) T. Oki, M. Suzuki, Y. Nishioka, A. Yasuda, K. Umegaki, and S. Yamada, *J. Urol.*, 173, 1395-1399 (2005)

4) F. Debruyne, P. Boyle, F. Calais Da Silva, J. G. Gillenwater, F. C. Hamdy, P. Perrin, P. Teillac, R. Vela-Navarrete, J. P. Raynaud, and C. C. Schulman, *Eur. Urol.*, 45, 773-780 (2004)

5) J. C. Carraro, J. P. Raynaud, G. Koch, G. D. Chisholm, F. D. Silverio, P. Teillac, F. Calais Da Silva, J. Cauquil, D. K. Chopin, F. C. Hamdy, M. Hanus, D. Hauri, A. Kalinteris, J. Marencak, A. Perier, and P. Perrin, *Prostate*, 29, 231-240 (1996)

6) B. J. Gurley, S. F. Gardner, M. A. Hubbard, D. K. Williams, W. B. Gentry, J. Carrier, I. A. Khan, D. J. Edwards, and A. Shah, *Clin. Pharmacol. Ther.*, 76, 428-440 (2004)

7) 日本クリニカルエビデンス編集委員会，クリニカルエビデンス日本語版，日経BP社，東京，2004：pp1120-1121

8) P. R. Solomon, F. Adams, A. Silver, J. Zimmer, and R. De Veaux, *JAMA*, 288, 835-840 (2002)

9) T. Sugiyama, Y. Kubota, K. Shinozuka, S. Yamada, K. Yamada, and K. Umegaki, *Food Chem. Toxicol.*, 42, 953-957 (2004)

10) M. Smith., K. M. Lin, and Y. P. Zheng, *Clin. Pharmacol. Ther.*, 69, PIII-89 (2001)

11) B. J. Gurley., S. F. Gardner, M. A. Hubbard, D. K. Williams, W. B. Gentry, Y. Cui, and C. Y. W. Ang, *Clin. Pharmacol. Ther.*, 72, 276-287 (2002)

12) J. Rowin., and S. L. Lewis, *Neurology.*, 46, 1775-1776 (1996)

13) M. Rosenblatt., and J. Mindel, *N. Eng. J. Med.*, 336,1108 (1997)

14) M. K. Matthews, Jr, *Neurology*. 50, 1933-1934 (1998)

15) J. Engelsen., J. D. Nielsen, and K. Winther, *Thromb. Haemost.*, 87, 1075-1076 (2002)

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〈抄録〉第26回 日本臨床薬理学会年会　2005年12月1～3日　別府
シンポジウム10：代替医療と臨床薬理

4．メディカルハーブの薬効解析と臨床薬との相互作用

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近年、代替医療の普及と共に、健康増進や疾患の予防・治療を目的に自然食品や健康食品への関心が高まっている。特に高齢者では、医薬品とともに健康食品の摂取率が高く、この傾向は今後益々増加すると予想される。一方、健康食品の過剰摂取や医薬品との相互作用による有害事象が報告され、それらの有効性および安全性の検証が重要となっている。欧米で民間薬として伝承されてきたメディカルハーブは様々な疾患に広く利用され続け、本邦でも健康食品として販売されている。特に高齢者に服用率が高いイチョウ葉、ノコギリヤシ果実およびセントジョーンズワートの各エキスは、欧州ではそれぞれ血液循環障害や老年性痴呆，前立腺肥大症に伴う排尿障害症状ならびにうつ症状の改善・治療薬として処方されている。我々はハーブ類の有効性、安全性および臨床薬との相互作用・併用効果を検証した。

イチョウ葉エキス (Ginkgo biloba Extract: GBE): GBEを反復経口投与した老齢ラットにおいて、肝薬物代謝酵素のチトクローム P450(CYP)の誘導作用に加え、抗糖尿病薬のトルブタミド経口投与後の血糖低下作用が対照群に比べ有意に減弱した(Fig. 1)。[1,2] 一方、GBE の単回投与ではトルブタミドによる血糖低下作用が増強され、CYP2C9 の競合的拮抗によるトルブタミド代謝の阻害が示唆された。次に、健常人に GBE(360 mg)を 28 日間反復経口投与することにより、トルブタミドの血漿中濃度-時間曲線下面積(AUC)及び血漿中トルブタミドと代謝物の AUC 比はいずれも投与前と比べ有意に減少し、血糖低下作用も減少した。一方、CYP3A4 の基質である鎮静薬のミダゾラム経口投与後の AUC は GBE 投与前に比べ有意に増加し、経口クリアランス(Cltot/F)は有意に減少した。これより、GBE は臨床において CYP2C9 や CYP3A4 の基質となる医薬品との相互作用を起こす可能性が示唆され、併用には注意が必要であると考えられた。

ノコギリヤシ果実エキス (Saw Palmetto Extract: SPE): SPE は、酢酸誘発頻尿ラットのシストメトリーによる排尿パラメーターの解析から、十二指腸投与により排尿間隔を有意に延長し、一回排尿量を有意に増加することが示された(Fig. 2)。[3] この SPE の頻尿改善作用は正常ラットより酢酸誘発頻尿ラットで顕著であったことから、病態特異的であると考えられた。また、SPE は、ラジオレセプターアッセイ実験において、排尿障害の発症に関与する前立腺α_1受容体および膀胱ムスカリン性受容体に結合活性を示した。これより、SPE は一部、臨床で繁用されている排尿障害治療薬(α_1 遮断薬や抗コリン薬)と類似した作用機構により、前立腺肥大などが原因となって発症する閉塞性および刺激性排尿症状を改善することが考えられた。また、SPE の反復投与は、ラットにおける血液検査値および肝薬物代謝酵素活性に影響しなかったことから、GBE とは異なり医薬品との代謝過程における相互作用の可能性は少ないと考えられた。

セントジョーンズワートエキス (St John's Wort: SJW): 抗うつ作用を有する SJW は、マウス脳のセロトニン再取り込み部位への結合活性を示さず、脳神経終末分画におけるセロトニンの再取り込みを濃度依存的に抑制した。[4] また、SJW は選択的セロトニン再取り込み阻害薬の抗うつ作用を相乗的に増強した。これより、SJW の抗うつ作用機構は臨床で汎用される選択的セロトニン再取り込み阻害薬(SSRI)とは相異すると考えられた。

本研究結果より、ハーブ類の有効性、安全性および臨床薬との相互作用の科学的検証がその適正使用において極めて重要となることが示された。SPE のヒト組織受容

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体結合活性および有効成分の分離同定や、SJW と抗うつ薬との併用効果などについて詳細に検討している。また、α_1 遮断薬治療の前立腺肥大症患者における SPE などの健康食品の摂取状況や臨床薬との併用効果の調査研究を泌尿器科医と共同で進め、さらに健康食品の作用について positron emission tomography (PET)を用いた非侵襲的手法による新規解析法も考案している。ハーブ類由来創薬ならびに「薬食」を基盤とした新たな医療体系の構築を目指している。

文献

1) T. Sugiyama, Y. Kubota, K. Shinozuka, S. Yamada, K. Yamada, and K. Ukegaki: Induction and recovery of hepatic drug metabolizing enzymes in rats treated with Ginkgo biloba extract. Food and Chem. Toxicol., **42**, 953-957 (2004)
2) T. Sugiyama, Y. Kubota, K. Shinozuka, S. Yamada, J. Wu and K. Umegaki: Ginkgo biloba extract modifies hypoglycemic action of tolbutamide via hepatic cytochrome p450 mediated mechanism in aged rats. Life Sci., 75, 1113-1122 (2004)
3) T. Oki, M. Suzuki, Y. Nishioka, A. Yasuda, K. Umegaki and S. Yamada: Effects of Saw palmetto extract on micturition reflex of rats and its autonomic receptor binding activity. J. Urol., 173, 1395-1399 (2005)
4) K. Hirano, Y. Kato, S. Uchida, Y. Sugimoto, J. Yamada, K. Umegaki and S. Yamada: Effects of oral administration of extracts of Hypericum perforatum (St John's wort) on brain serotonin transporter, serotonin uptake and behaviour in mice. J. Pharm. Pharmacol., 56, 1589-1595 (2004)

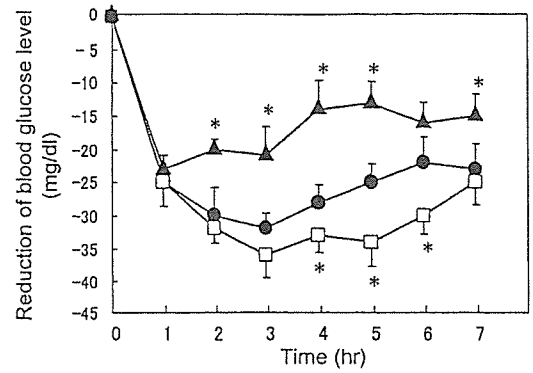

Fig. 1. Effects of simultaneous treatment and 5 day pretreatment with Ginkgo biloba extract (GBE) on the hypoglycemis effect of tolbutamide in aged rats. ●, control group; ▲, 5 days pretreatment group with GBE; □, simultaneous treatment group with GBE. Each point represents mean±SD from six rats.

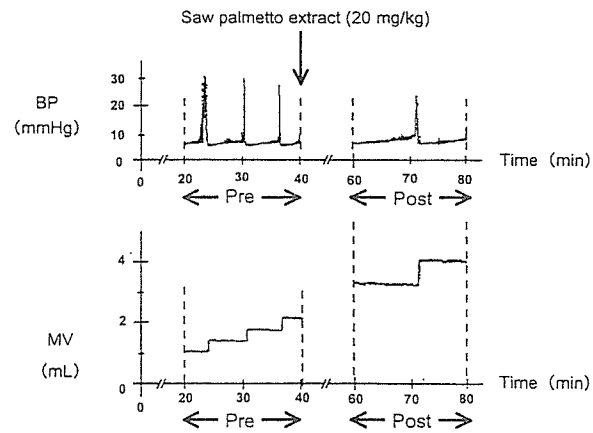

Fig. 2. Effects of intraduodenal administration of Saw Palmetto Extract (SPE: 20 mg/kg) on bladder pressure (BP) and micturition volume (MV) on cystometrograms of 0.1% acetic acid infused anesthetized rats. Pre: pre-treatment with SPE; Post: post-treatment with SPE.

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研究報告

特定保健用食品の組み合わせ摂取の有用性の検討
—ジアシルグリセロール十植物ステロール添加油(エコナ油)と食物繊維(コレスケア)の併用—

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はじめに

特定保健用食品(以下，特保食品)として数多くの商品が登場し，それぞれ有用性が確認されている．しかし，特保食品の利用者は，単独にて摂る場合も多いが，時として複数組み合わせて摂る場合もある．

特保食品の有用性は，それぞれ開発時に確認されているが，複数摂取する場合の有用性については十分な検証がなされておらず，かかる意味から，われわれは主として血清脂質に関与する食品を選び，アンケートの結果より多い組み合わせを選定し，組み合わせ摂取の有用性を検討してきた．そこで，ジアシルグリセロールと大豆蛋白の併用，ジアシルグリセロールとカテキン緑茶の併用，大豆蛋白とカテキン緑茶の併用などについて，その安全性，有効性を臨床例を用いて検討し報告してきた[1-3]．

今回，さらにジアシルグリセロール＋植物ステロール添加油にアルギン酸ナトリウムを中心として食物繊維を併用し，その有用性について検討したので，ここにその結果をまとめたい．

対象および方法

対象は，三越厚生事業団診療所に高血圧，あるいは高脂血症にて通院中の平均年齢60.3±11.4歳の男性4例，女性15例計19例である．一部はカルシウム拮抗薬，あるいはプラバスタチンにてコントロールされているが，血清コレステロール値はなお軽度に高値を維持し，安定している症例である．いずれにせよ，総コレステロール値が220 mg/dL以上で，当施設における倫理委員会にて了承されたプロトコルにつき，本試験の内容を説明し，理解されるとともに，参加の同意を文書にて提出している．

5％植物ステロール添加のジアシルグリセロール(以下，エコナ油：花王(株))1日10 g(小袋包装)を3カ月間摂取し，2カ月目にアルギン酸ナトリウム(以下，コレスケア：大正製薬(株))1缶(4 g)を1カ月間併用し，その後1カ月エコナ油10 g/日を摂取した．その際，確実に摂り得るように，小袋に正確に分包したものである．

エコナ油開始時，コレスケア併用時，エコナ油単独摂取に戻った時点，およびその1カ月摂取が終了したときに，必ず自覚症状を問い合わせるとともに，体重，BMI，体脂肪(%)，血圧，脈拍を測定した．

その際，空腹時採血を行い，総コレステロール，トリグリセライド，HDL-コレステロール，LDL-コレステロール(換算による)を測定した．さらに，血糖，アディポネクチン，マロンジアルデハイドLDL(MDA-LDL)，hs CRPも測定した．

また，安全性の指標として肝機能をAST(GOT)，ALT(GPT)，LDHにおいて測定し，腎機能をクレアチニン(Cr)，尿酸として測定し，筋肉障害についてCPK，および全般的指標として末梢血液(白血球，赤血球，ヘモグロビン，ヘマトクリット，MCV，MCH，MCHC，血小板数)を測定した．

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表1　ジアシルグリセロール十植物ステロール添加油(エコナ油)，
食物繊維(コレスケア)摂取の影響(n=19)

	前	エコナ	エコナ+コレスケア	エコナ
体重(kg)	60.4±9.1	60.6±9.2	60.1±9.1	60.3±9.5
体脂肪(%)	28.1±6.2	28.3±6.3	29.0±6.8	30.1±5.6
BMI	24.3±3.0	24.3±2.9	24.2±3.0	24.2±3.2
血圧　収縮期(mmHg)	126.6±10.6	127.5±13.3	129.6±10.2	124.7±11.8
拡張期(mmHg)	80.6±6.9	78.1±8.2	80.7±6.8	76.9±10.7
脈拍(拍/分)	64.0±2.8	64.0±2.8	64.3±2.6	63.7±3.0

男性：4例，女性：15例
平均年齢：60.3±11.4歳

表2　血中脂質その他代謝物への影響

	前	エコナ	エコナ+コレスケア	エコナ
TC (mg/dL)	249.7±31.3	237.4±22.2	242.5±23.7	251.4±30.9
		*		
TG (mg/dL)	177.4±127.9	174.3±119.3	149.1±78.9	153.5±94.6
LDL-C (mg/dL)	154.8±25.2	142.6±16.1	149.1±18.3	155.7±26.8
		*		
HDL-C (mg/dL)	60.7±13.9	61.1±13.4	63.6±12.7	66.0±14.5
				**
Glu (mg/dL)	98.1±9.7	98.1±11.2	97.2±7.1	96.9±8.6
Adip (μg/mL)	10.93±5.2	10.6±5.0	12.1±5.1	12.7±6.5
			**	
				*
MDA-LDL(U/L)	176.4±62.2	191.0±65.7	180.9±53.1	180.7±60.7
hs CRP (mg/L)	1.0±0.72	0.84±0.62	0.87±0.69	0.84±0.6

*：$p<0.05$，**：$p<0.01$

成　　績

1．有効性について

表1に期間中の体重，体脂肪(%)，BMIの他，血圧，脈拍についての経過を示してある．体重にはほとんど変化を認めず，体脂肪率，BMIにも期間中の変動は認められていない．この状況は，血液中の脂質など代謝産物を検討するには好条件であるといえよう．また，収縮期，拡張期血圧，脈拍にも変化は認められていない．

表2に，血中脂質，その他代謝物の変動を示してある．総コレステロール，LDL-コレステロールはエコナ油摂取時に有意に減少を示している．コレスケア併用時では前値に比し総コレステロール，LDL-コレステロールのさらなる低下は認められていない．個々の症例をみると，6例は明らかな減少傾向がみられており，症例により異なるものと思われる．しかし，トリグリセライドに対しては，エコナ油にコレスケア併用時が最も低い．しかも，HDL-コレステロールはエコナ油とコレスケア併用で高くなり，エコナ油単独摂取時に最も高値となっている．同様の傾向は，アディポネクチンにおいても認められ，エコナ油とコレスケアの併用で高くなり，最終的なエコナ油単独摂取でも高値を保っている．

血糖，MDA-LDLには有意な変動は認められていない．hs CRPは1カ月後から低下の傾向があり，その状態が維持されている．

2．安全性について

表3に肝機能，腎機能，末梢血液所見についてまと

表3　肝，腎機能，末梢血液などに対する影響

	前	エコナ	エコナ＋コレスケア	エコナ
GOT (IU/L)	22.9±9.4	23.5±10.5	23.6±6.0	23.8±6.4
GPT (IU/L)	30.7±12.2	32.5±18.1	30.6±11.2	28.9±12.5
LDH (IU/L)	362.8±54.6	366.6±60.7	378.1±60.6	366.9±51.3
CPK (IU/L)	116.2±44.5	114.5±42.5	118.9±49.6	114.9±43.4
Cr (mg/dL)	0.78±0.23	0.73±0.20	0.60±0.15	0.63±0.18
		*	***	
			***	
				***
UA (mg/dL)	4.56±1.23	4.47±1.19	4.17±1.08	4.31±1.05
			**	
WBC (10^2/UL)	53.0±10.3	54.1±12.0	52.5±9.1	52.6±9.0
RBC (10^4/UL)	447.1±41.0	446.3±39.1	445.5±38.1	446.9±37.9
Hb (g/dL)	13.7±1.3	13.7±1.3	13.6±1.3	13.6±1.1
Ht (%)	40.7±3.4	40.8±3.5	40.7±3.1	40.9±3.0
MCV (fl)	91.0±2.8	91.4±2.9	91.3±2.6	91.5±3.0
MCH (pg)	30.7±1.2	30.7±1.3	30.6±1.1	30.5±1.0
MCHC (%)	33.7±0.8	33.6±0.8	33.5±0.9	33.3±0.6
PLT (10^4/UL)	24.9±3.6	26.1±5.1	25.8±4.5	26.6±4.5

*：$p<0.05$，**：$p<0.01$，***：$p<0.001$

めてある．

GOT，GPT，LDHは全期間を通じてほとんど変動は認められていない．CPKについても，同様にほとんど変動は認められていない．

Crは次第に減少しており，特にエコナ油とコレスケア併用時に最も低い値を示しており，尿酸についてもほぼ同様の結果である．

末梢血液所見については，白血球，赤血球，ヘモグロビン，ヘマトクリットも特に臨床的に問題となる変化は認められていない．また，MCV，MCH，MCHCなども臨床的に問題となる変動はみられていない．また，血小板数については，試験期間中，次第に増加する傾向がみられているが，臨床的には問題とならない．

考　　案

19例の軽度，中等度の高コレステロール血症例に試験開始時1カ月5％植物ステロール添加エコナ油を服用し，総コレステロールで5％，LDL-コレステロールで8％の有意の減少を認めている．これにコレスケアを併用した際に，総コレステロール，LDL-コレステロールは，さらに有意な減少を認めることはないが，トリグリセライドが16％の減少を認めている．その際，HDL-コレステロールは5％の増加をみるとともに，アディポネクチンは11％の上昇を認めている．すなわち，併用による動脈硬化のリスクの減少は明らかである．炎症マーカーであるhs CRPも軽度ながら13％の減少をみており，臨床的に長期にわたる観察を行う価値のあるものと考えられる．

アディポネクチン濃度は測定し得た16例中11例に，併用により上昇を認めている．アディポネクチンの低値が心疾患イベントのリスクとなることが確認されており[5]，この状態を改善し得る可能性のあることは注目しなければならない．

しかも，LDL-コレステロールのわずかの減少と，HDL-コレステロールの上昇，hs CRPの軽度の低下を伴っていることから，エコナ油とコレスケアの組み合わせ摂取の新しい有効性は評価されなければならない．

安全性についても，Cr，尿酸の低下を有意に認めており[6]，そのメカニズムは現段階では不明であるが，興味ある所見である．他の安全性の評価項目には，特に異常はみられないことも含めて，エコナ油とコレスケアの併用の有用性は高いものと考えられる．

おわりに

軽度，中等度の高コレステロール血症19例に，植物ステロール添加ジアシルグリセロール1日10 g連日摂取を続けながら，1カ月後アルギン酸ナトリウムの食物繊維4 gを併用することにより，総コレステロール，LDL-コレステロールの軽度の低下，トリグリセライドの減少傾向を認め，HDL-コレステロールの軽度の上昇を認めた．

さらに，アディポネクチンの有意の上昇と，炎症マーカーであるhs CRPの軽度の減少を認めた．

肝機能，腎機能，末梢血液など安全性の評価には異常は認められず，むしろクレアチニン，尿酸の低下を認めている．

以上のごとく，両特保食品の併用は有用性の高いものと考えられる．

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文　献

1）嘉津山ひとみ，山下　毅，中村治雄ほか：特定保健用食品の組み合わせ摂取による有効性，安全性の研究—エコナ油と大豆蛋白の併用—．Prog Med 2002；**22**：2782-2785.

2）嘉津山ひとみ，山下　毅，中村治雄ほか：特定保健用食品の組み合わせ摂取による安全性，有効性の検討Ⅱ—エコナ油とヘルシア緑茶の併用—．Prog Med 2004；**24**：841-844.

3）宮島恵美子，山下　毅，中村治雄ほか：特定保健用食品の組み合わせ摂取の有用性の検討—大豆蛋白と高濃度カテキン茶の併用—．Prog Med 2005；**25**：831-835.

4）Pischon T, Girman CJ, Hotamisligil GS, et al：Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 2004；**291**：1730-1737.

5）Van der Vleuten GM, van Tits LJH, den Heijer M, et al：Decreased adiponectin levels in familial combined hyperlipidemia patients contribute to the atherogenic lipid profile. J Lipid Res 2005；**46**：2397-2404.

6）Milionis HJ, Kalantzi KJ, Goudevenos JA, et al：Serum uric acid levels and risk for acute ischaemic non-embolic stroke in elderly subjects. J Intern Med 2005；**258**：435-441.

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Note

Evaluation of the Correlation Between Amount of Curcumin Intake and its Physiological Effects in Rats

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We examined the correlation between amount of curcumin intake and its physiological effects on indices of liver function, serum and liver lipid profiles in rats. Animals were fed diets containing 0.5, 5 and 50 mg curcumin per 100 g body weight for 28 days.

HDL-cholesterol concentrations of rats fed curcumin diets were significantly higher ($P<0.05$) than those of the control group, and serum TG concentration of rats fed the x100 curcumin diets was significantly lower ($P<0.05$) than that of the x1 curcumin group. Serum TG concentration of rats fed curcumin diets tended to decrease in a curcumin dose-dependent manner. These results indicate that curcumin intake can improve serum lipid profiles effectively.

Keywords: Curcumin, Rat serum, Triglyceride, HDL-cholesterol, Liver function.

Introduction

Turmeric has long been used as a traditional remedy in Asia. Its commercial derivatives currently demand high prices in the so-called health food market of Japan because it is well known that they have various beneficial effects on human health. These physiological effects are emphasized by manufacturers and promote sales in the so-called health food market.

The main physiological ingredient of turmeric is considered to be the 3–5% of curcumin contained in *Curcuma longa*. Curcumin is a well-known natural anti-oxidant (Sharma, 1976). In addition to such a function, it has been reported that curcumin has various physiological functions such as lowering cholesterol (Ramirez-Tortosa *et al.*, 1999), improving liver function (Park *et al.*, 2000), suppressing tumor activity (Surh, 2002), and it can be used as an anti-inflammatory (Rao *et al.*, 1982).

Although curcumin is thought to be the main active ingredient of turmeric, whether there is a clear relation between the physiological function of turmeric and curcumin content has not yet been established. This is because turmeric consists of various natural materials such as minerals, dietary fiber, tannin, curcumin, flavonoids, camphor, azulene and similar compounds. In addition, the curcumin content in turmeric is about 5% at most (Hiserodt *et al.*, 1996), and absorptivity is considered to be very low (Asai and Miyazawa, 2000). Therefore, it seems unlikely that curcumin acts independently when turmeric is ingested because similar effects are observed in *Curcuma aromatica* (Salisb) and *Curcuma zedoaria* (Roscoe), which hardly contain curcumin. Thus, the relation between the amount of curcumin contained in turmeric and substantial physiological effects are still poorly understood. Therefore, it is important to examine the actual effects associated with ingestion of a certain amount or excessive intake of curcumin.

In the current study, to investigate the relation between amount of curcumin intake and its physiological effects on indices of liver function, serum and liver lipid profiles and other biochemical parameters, male Wistar rats were fed 0.5 mg (x1), 5 mg (x10), 50 mg (x100) curcumin-containing diets or a curcumin-free diet for 28 days. In addition, we also conducted histological observations of metabolic organs such as liver and kidney to gain more insight into the metabolic state resulting from excessive curcumin.

Materials and Methods

Male Wistar rats (8 weeks old) were purchased from Japan SLC Inc. (Shidzuoka, Japan). After acclimation for 1 week, rats were randomly divided into four groups ($n=6$/group). Rats were fed experimental diets ad libitum for 4 weeks. Experimental diets were based on AIN-93G formula of the following ingredients (g/kg diet): casein, 200; corn starch, 150; test oil, 100; AIN-93G mineral mixture, 35; AIN-93G vitamin mixture, 10; cellulose, 50; D, L-methionine, 3; choline bitartrate, 2 and ~1000 sucrose. The experimental diets contained 0.5 (x1), 5 (x10) or 50 (x 100) mg curcumin per 100 g body weight, while the control group received the AIN-93G type diet without curcumin. The reagent-grade curcumin was purchased from Wako Pure Chemical Industries, Ltd. The basal daily amount of curcumin in rats was estimated by weight conversion based on the recommended daily intake for humans. At the end of the experimental period, after overnight fast-

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ing, rats were anesthetized and sacrificed for analysis. Care and use of laboratory animals was in accordance with the guidelines of the National Institute of Health and Nutrition.

Blood samples were obtained from the rat abdominal aorta after overnight fasting, and centrifuged at 3,000 rpm for 15 min. Sera were stored at −80℃ before analysis. Serum lipids (total cholesterol, high density lipoprotein (HDL)-cholesterol and triglyceride (TG)), liver function indices (GOT, GPT, γ-GPT, ALP and LDH) and other biochemical parameters (total protein and blood glucose) were analyzed enzymatically using commercially available assay kits (Wako Pure Chemical, Osaka). Serum insulin concentrations were measured using a commercially available EIA kit (Biotrak, Amersham Pharmacia Biotech, MO).

Liver was excised, weighed and stored at −80℃ until analysis. The liver lipids were extracted with chloroform-methanol (2: 1 v/v) (Folch *et al.*, 1957). Liver cholesterol and TG concentrations were determined using a Cholesterol E-test and Triglyceride E-test (Wako, Osaka) as described elsewhere with minor modifications (Carr *et al.*, 1993).

The liver and kidneys obtained from rats fed control and x100 curcumin diets were fixed with 10% formaldehyde solution (pH=7.4) and embedded in paraffin. Paraffin-embedded specimens were prepared, and stained with hematoxylin and eosin. Sample preparation and microscopic examination was performed at the Sapporo Pathology Research Institute.

Data are presented as means±SEM (standard error of the mean). The statistical significance of the difference was evaluated by ANOVA followed by Fisher's PLSD test. Differences at $P<0.05$ were considered to be significant.

Results and Discussion

There were no significant differences in body weight gain, food intake, and relative weights of liver, kidney, spleen, testis, epididymal and perirenal adipose tissue between groups by curcumin intake. These findings indicate that excessive curcumin intake did not affect the growth of rats. Therefore, it is thought that curcumin as a component of food is not harmful on rat growth in the range of curcumin consumption measured in the present study. Pathological examination revealed lipid deposition or extramedullary hematopoiesis in one sample and small granuloma in the livers of two rats fed the x100 curcumin diet. However, these manifestations were not considered a pathological problem.

Serum HDL-cholesterol concentrations of rats fed curcumin diets were significantly higher ($P<0.05$) than that of control group. Serum TG concentration of rats fed the x100 curcumin diets was significantly lower ($P<0.05$) than that of the x1 curcumin group. Serum TG concentration tended to decrease in a curcumin dose-dependent manner (Fig. 1). No significant differences in serum and liver cholesterol, and liver TG concentrations were observed between groups. The hypocholesterolemic effect of curcumin can probably be explained by its effect

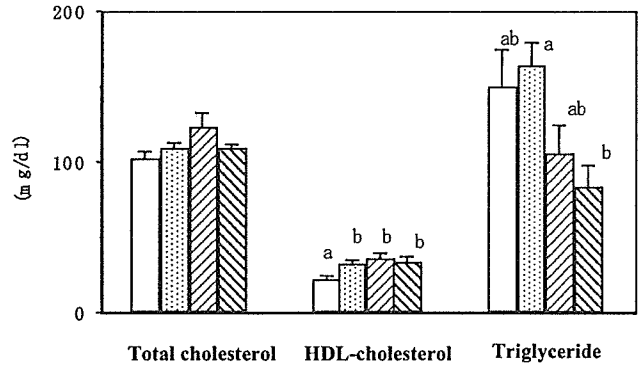

Fig. 1. Serum lipid profiles of rats maintained on different curcumin diets.
Values are means±SEM for 6 rats. Values not sharing a common letter differ, $P<0.05$.
□: Control diet, ⬚: x1 Curcumin diet, ▨: x10 Curcumin diet, ▧: x100 Curcumin diet.

on the stimulation of bile fluid and biliary cholesterol secretion and enhanced excretion of bile acids and cholesterol in feces (Ramprasad and Sirsi, 1957; Patil and Srinivasan, 1971; Srinivasan and Sambaiah, 1991). Although, in this study, we did not observe a marked reduction in serum cholesterol concentrations in rats fed curcumin diets, the results agree with a previous report, which indicated that the plasma cholesterol levels of animals fed cholesterol-free diet were not affected by curcumin intake (Rao *et al.*, 1970). Furthermore, curcumin ingestion contributed to an increase in HDL-cholesterol concentration regardless of the amount of curcumin ingestion. These results indicate that curcumin intake modulated HDL- and LDL-cholesterol concentrations, maintaining proportions at desirable levels. It is known that a decrease in the ratio of LDL- to HDL-cholesterol concentrations leads to improvement in the arteriosclerosis index (Hostmark *et al.*, 1990). Moreover, although Asai *et al.* also observed a reduction in serum TG concentration (Asai and Miyazawa, 2001), the critical regulatory mechanism of curcumin on the reduction in serum TG concentration has not yet been clarified. Thus, the alterations in serum lipid profiles observed in the present study could not be explained by the increase in fecal bile acid excretion alone, and further detailed study will be required to examine the regulatory mechanism of curcumin on serum lipid profiles.

Liver function indices were not significantly influenced by curcumin ingestion, although GOT and GPT slightly increased, and γ-GTP and ALP tended to decrease in accordance with an increase in curcumin intake (Table 1). It is well known that effects of liver function improvement are the typical physiological effect associated with curcumin (Nirmala and Puvanakrishnan, 1996; Rukkumani *et al.*, 2004). Since the present study was carried out using a normal animal model, a marked improvement in liver function might not be observed under these experimental conditions. However, use of an impaired liver function animal model, a heavy cholesterol diet, or over-

Table 1. Serum biochemical parameters in rats maintained on different curcumin diets.

	Control	Curcumin		
		x1	x10	x100
		(IU/L)		
GOT	41.2±4.54	54.9±2.25	49.7±1.84	52.8±5.26
GPT	1.57±0.45	3.53±0.53	0.47±0.30	2.59±0.59
γ-GTP	6.11±0.76	5.67±0.51	4.11±0.25	3.93±0.47
		(K-A unit)		
ALP	88.5±13.6	63.9±8.28	56.1±10.1	57.2±4.68
		(mg/dl)		
LDH	736.2±3.23	727.9±5.99	731.1±6.66	672.2±67.3
		(g/dl)		
Total protein	6.33±0.20	6.16±0.11	5.84±0.18	5.97±0.06
		(mg/dl)		
Blood glucose	96.9±12.9a	120.6±4.89ab	140.6±8.68bc	159.0±9.00c
		(ng/ml)		
Insulin	1.13±0.11	1.11±0.18	1.75±0.34	2.36±0.63

Values are means±SEM, n=6. Values not sharing a common letter differ significantly ($P<0.05$).

dose of curcumin under the cruel experimental conditions in animal models might be better suited for illustrating the effect of the amount of curcumin intake on improved liver function (Park *et al.*, 2000; Patil and Srinivasan, 1971; Rao *et al.*, 1970). In the present study, however, we found that moderate amounts of curcumin consumption improved the ratio of HDL- and LDL-cholesterol concentrations, even in a healthy animal model. While curcumin intake increased both blood glucose and serum insulin concentrations in this study (Table 1), previous studies on blood glucose, serum insulin or diabetes found that the antioxidant properties of curcumin had antidiabetic effects (Srivivasan *et al.*, 2003; Arun and Nalini, 2002; Nishizono *et al.*, 2000). It may therefore be necessary to consider changes in these biochemical characteristics in future research.

In conclusion, it is noteworthy in this study that curcumin intake improved the proportion of HDL- and LDL-cholesterol concentrations, even at curcumin concentrations found in turmeric, and excessive curcumin intake reduced the serum TG concentration in healthy rats. These results imply that curcumin may contribute to the regulation of lipid metabolism. The mechanisms of action are not yet clear and further study will be necessary to understand how curcumin affects liver function. In addition, the physiological effect of other turmeric ingredients, such as natural polyphenolic compounds, needs to be considered.

To clarify the substantial physiological benefits of turmeric or curcumin, we intend to study the effects of long-term or excessive feeding in an impaired animal model, such as animals with liver dysfunction and hyperlipidemia. A series of such experiments will increase our understanding of the regulatory mechanisms of lipid metabolism and the correlation between physiological function and amount of turmeric intake.

References

Arun, N. and Nalini, N. (2002). Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats. *Plant Foods Hum. Nutr.*, **57**, 41-52.

Asai, A. and Miyazawa, T. (2000). Occurrence of orally administered curcuminoid as glucuronide and glucuronide/sulfate conjugates in rat plasma. *Life Sciences*, **67**, 2785-2793.

Asai, A. and Miyazawa, T. (2001) Dietary curcuminoids prevent high fat diet-induced lipid accumulation in rat liver and epididymal adipose tissue. *J. Nutr.*, **131**, 2932-2935.

Carr, T.P., Andresen, C.J. and Rudel, L.L. (1993). Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. *Clin. Biochem.*, **26**, 39-42.

Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biological Chemistry*, **226**, 497-509.

Hiserodt, R., Hartman, T.G., Ho, C.T. and Posen, R.T. (1996). Characterization of powdered turmeric by liquid chromatography-mass spectrometry and gas chromatograph-mass spectrometry. *J. Chromatogr.*, **740**, 51-63.

Hostmark, A.T., Osland, A., Simonsen, S. and Levorstad, K. (1990). Lipoprotein-related coronary risk factors in patients with angiographically defined coronary artery disease: relation to number of stenosed arteries. *J. Intern. Med.*, **228**, 317-21.

Nirmala, C. and Puvanakrishnan, R. (1996). Protective role of curcumin against isoproterenol induced myocardial infarction in rats. *Mol. Cell Biochem.*, **159**, 85-93.

Nishizono, S., Hayami, T., Ikeda, I. and Imaizumi, K. (2000). Protection against the diabetogenic effect of feeding tert-butylhydroquinone to rats prior to the administration of streptozotocin. *Biosci. Biotechnol. Biochem.*, **64**, 1153-1158.

Park, E.J., Jeon, C.H., Ko, G., Kim, J., Sohn, D.H. (2000). Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. *J. Pharm. Pharmacol.*, **52**, 437 440.

Patil, T.N. and Srinivasan, M. (1971). Hypocholesterolemic effect

of curcumin in induced hypercholesterolemic rats. *Indian J. Exp. Biol.*, **9**, 167-169.

Ramirez-Tortosa, M.C., Mesa, M.C., Aguilera, M.C., Quiles, J.L., Baro, L., Ramirez-Tortosa, C.L., Martinez-Victoria, E. and Gil, A. (1999). Oral administration of a turmeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis. *Atherosclerosis*, **147**, 371-378.

Ramprasad, C. and Sirsi, M. (1957). Curcuma logna and bile secretion — Quantitative changes in the bile constituents induced by sodium curcuminate. *J. Sci. Industr. Res.*, **16**, 108-110.

Rao, D.S., Sekhara, N.C., Satyanarayana, M.N. and Srinivasan, M. (1970). Effect of curcumin on serum and liver cholesterol levels in the rats. *J. Nutr.*, **100**, 1307-1316.

Rao, T.S., Basu, N. and Siddiqui, H.H. (1982). Anti-inflammatory activity of curcumin analogues. *Indian J. Med. Res.*, **75**, 574-578.

Rukkumani, R., Aruna, K., Varma, P.S., Rajasekaran, K.N. and Menon, V.P. (2004). Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. *J. Pharm. Pharm. Sci.*, **7**, 274-283.

Sharma, O.P. (1976). Antioxidant activity of curcumin and related compounds. *Biochem. Pharmacol.*, **25**, 1811-1812

Srivivasan, A., Menon, V.P., Periaswamy, V. and Rajasekaran, K. N. (2003). Protection of pancreatic beta-cell by the potential antioxidant bis-o-hydroxycinnamoyl methane, analogue of natural curcuminoid in experimental diabetes. *J. Pharm. Pharm. Sci.*, **6**, 327-333.

Srinivasan, K. and Sambaiah, K. (1991). The effect of spices on cholesterol 7 alpha-hydroxylase activity and on serum and hepatic cholesterol levels in rats. *Int. J. Vitam. Nutr. Res.*, **61**, 364-369.

Surh, Y.J. (2002). Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. *Food Chem. Toxicol.*, **40**, 1091-1097.

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Selective protection of curcumin against carbon tetrachloride-induced inactivation of hepatic cytochrome P450 isozymes in rats

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Abstract

We investigated the effects of curcumin, a major antioxidant constituent of turmeric, on hepatic cytochrome P450 (CYP) activity in rats. Wistar rats received curcumin-containing diets (0.05, 0.5 and 5 g/kg diet) with or without injection of carbon tetrachloride (CCl_4). The hepatic CYP content and activities of six CYP isozymes remained unchanged by curcumin treatment, except for the group treated with the extremely high dose (5 g/kg). This suggested that daily dose of curcumin does not cause CYP-mediated interaction with co-administered drugs. Chronic CCl_4 injection drastically decreased CYP activity, especially CYP2E1 activity, which is involved in the bioactivation of CCl_4, thereby producing reactive free radicals. Treatment with curcumin at 0.5 g/kg alleviated the CCl_4-induced inactivation of CYPs 1A, 2B, 2C and 3A isozymes, except for CYP2E1. The lack of effect of curcumin on CYP2E1 damage might be related to suicidal radical production by CYP2E1 on the same enzyme. It is speculated that curcumin inhibited CCl_4-induced secondary hepatic CYPs damage through its antioxidant properties. Our results demonstrated that CYP isozyme inactivation in rat liver caused by CCl_4 was inhibited by curcumin. Dietary intake of curcumin may protect against CCl_4-induced hepatic CYP inactivation via its antioxidant properties, without inducing hepatic CYPs.

Keywords: Curcumin; Carbon tetrachloride; Free radicals; CYP2E1; Hepatotoxicity

Introduction

Recently, interest in complementary and alternative medicine has grown rapidly in industrialized countries, and the demand for herbal remedies has currently increased (De Smet, 2002; Ammon and Wahl, 1991). Turmeric, the rhizome of *Curcuma longa* L., has traditionally been used for treatment of gastrointestinal colic, flatulence, hemorrhage, hematuria, menstrual difficulties and jaundice. The anti-inflammatory and hepatoprotective characteristics of turmeric and its constituents have been widely investigated (Govindarajan, 1980; Luper, 1999; Miquel et al., 2002). The most well-researched component of turmeric is curcumin (diferuloylmethane, Fig. 1). Curcumin is the major yellow pigment comprising 3–6% of turmeric, and has been widely used in curry, mustard, cosmetics and drugs (Govindarajan, 1980; Miquel et al., 2002). Curcumin is well known for its pharmacological properties including antioxidant, anti-inflammatory, antimutagenic and anticancer activity (Miquel et al., 2002; Okada et al., 2001; Asai and Miyazawa, 2001; Ramirez-Tortosa et al., 1999; Sharma et al., 2004). The preventive and improved effects of curcumin on symptoms of liver diseases are shown to stem from its antioxidant effects (Rukkumani et al., 2004; Park et al., 2000; Nanji et al., 2003).

Many alternative remedies including turmeric may be taken with medicine; hence, pharmacological interactions are a concern in clinical therapy (Ernst, 2002; Williamson, 2001). Changes in the pharmacokinetics and pharmacodynamics of co-administered drugs affects clinical efficacy, and occasion-

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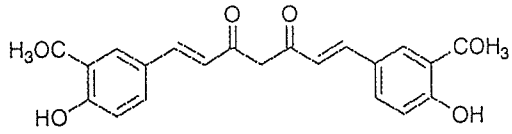

Fig. 1. Chemical structure of curcumin.

ally severe adverse reactions occur. We have examined cytochrome P450 (CYP)-mediated interactions with herbal remedies and medication (Umegaki et al., 2002; Kubota et al., 2004; Sugiyama et al., 2004). Regarding the metabolic functions of the liver, the effects of curcumin on metabolic enzymes, especially hepatic CYP activity, have not been completely elucidated. In addition, many liver diseases are related to lipid peroxidation in liver tissue, and some antioxidant components have a protective effect against liver damage. Curcumin has antioxidative properties and prevents some oxidative stress, and the action of curcumin has been shown to be beneficial for inhibition of tissue injury (Luper, 1999; Miquel et al., 2002; Okada et al., 2001; Khopde et al., 2000). Carbon tetrachloride (CCl_4), a well-known model compound for producing chemical hepatic injury, requires biotransformation by hepatic microsomal CYP to produce toxic metabolites, namely trichloromethyl free radicals (Recknagel et al., 1989; Brattin et al., 1985; Brautbar and Williams, 2002). CYP2E1 is the major isozyme involved in bioactivation of CCl_4 and subsequent production of free radicals (Recknagel et al., 1989). It has been proposed that the antioxidative action of curcumin plays an important role in its hepatoprotective effects against CCl_4-induced liver injury (Park et al., 2000). However, the mechanism by which curcumin protects the liver against CCl_4-induced toxicity is unclear, particularly in association with CYP activity.

This study was undertaken to evaluate the effect of repeated curcumin ingestion on hepatic CYP enzymes and to examine the protective effect of curcumin on CCl_4-induced hepatic CYP damage in rats.

Materials and methods

Materials

Curcumin was purchased from Wako Pure Chemical Ltd. (Osaka, Japan). Resorufin, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, testosterone, 6β-hydroxytestosterone, corticosterone, *p*-nitrophenol, 4-nitrocatechol and 7-ethoxycoumarin were purchased from Sigma (St. Louis, MO, USA). (*S*)-Warfarin and 7-hydroxywarfarin were obtained from Ultrafine (Manchester, England). NADPH was obtained from Oriental Yeast (Tokyo, Japan). Other reagents were obtained from Wako Pure Chemical Ltd. (Osaka, Japan).

Animal experiments

Male Wistar rats (5 weeks old) obtained from Japan SLC (Shizuoka, Japan) were housed individually in stainless steel, wire-bottomed cages at a constant temperature (23 ± 1 °C) under a 12 h light–dark cycle. Rats were given AIN-93G based diets (containing 53.2% (w/w) α-corn starch, 20% milk casein, 10% sucrose, 7% corn oil, 5% cellulose, 3.5% mineral mix (AIN-93G-MX), 1.0% vitamin mix (AIN-93G-VX), 0.3% L-cysteine and 0.0014% *tert*-butylhydroquinone) (Reeves et al., 1993) with or without curcumin (0.05, 0.5 and 5 g/kg diet) for 4 consecutive weeks. In order to examine the protective effects of curcumin on the liver damage, rats were subcutaneously injected with CCl_4 (50% (v/v in olive oil) for 0.2 ml/100 g body weight) twice a week during the 7 weeks of curcumin ingestion. After these treatments, rats were anesthetized with pentobarbital and sacrificed, the blood was collected, and the livers were immediately removed and weighed. The glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities in plasma were determined using an assay kit, transaminase CII-Test Wako (Wako Pure Chemical Ltd., Osaka, Japan).

All procedures were in accordance with the National Institute of Health and Nutrition guidelines for the Care and Use of Laboratory Animals.

Preparation of microsome and cytosolic fractions from the liver

The liver was rinsed with 0.9% (w/v) NaCl solution and homogenized in 50 mmol/L Tris–HCl buffer (pH 7.4) containing 0.25 mol/L sucrose. The homogenate was centrifuged at 10,000×*g* at 4 °C for 30 min. The supernatant was further centrifuged at 105,000×*g* at 4 °C for 60 min. The supernatant was used as the cytosolic fraction for the assay of glutathione *S*-transferase, the activity of which was determined using 1-chloro-2,4-dinitrobenzene as a substrate (Habig and Jakoby, 1981). The pellet was washed once with 50 mmol/L Tris–HCl buffer (pH 7.4) containing 0.25 mol/L sucrose by centrifugation at 105,000×*g* at 4 °C for 60 min, and the concentration and activities of CYP were analyzed.

Protein concentrations of microsomal and cytosolic fractions were determined using a BCA protein assay kit (Pierce, Rockford, IL, USA).

Analysis of CYP enzyme activities

The CYP content was quantified by the method of Omura and Sato (1964). The activities of various CYP enzymes were determined by HPLC methods as reported previously (Umegaki et al., 2002). The subtypes of CYP enzymes examined and the corresponding CYPs were ethoxyresorufin *O*-deethylase, CYP1A1; methoxyresorufin *O*-demethylase, CYP1A2; pentoxyresorufin *O*-dealkylase, CYP2B; (*S*)-warfarin 7-hydroxylase, CYP2C9; *p*-nitrophenol hydroxylase, CYP2E1; and testosterone 6β-hydroxylase, CYP3A (Hanioka et al., 2000; Mishin et al., 1996; Lang and Bocker, 1995).

Statistical analysis

The data are presented as means with standard deviation (S.D.) for the individual groups. Statistical analysis of the data was carried out using ANOVA followed by a post hoc test of Fisher's

Table 1
Effects of curcumin on the weights of body and liver, and hepatic drug metabolizing enzymes in rats

	Untreated control	Curcumin		
		(0.05 g/kg)	(0.5 g/kg)	(5 g/kg)
Body weight (g)	211.8±9.9	216.1±6.7	215.1±5.5	219.9±9.6
Liver weight (%/body weight)	2.90±0.08	2.93±0.13	3.02±0.11	3.04±0.09
Hepatic metabolizing enzymes				
Cytochrome P450 content (nmol/mg protein)	0.615±0.077	0.584±0.088	0.686±0.044	0.675±0.055
Glutathione *S*-transferase (μmol/mg protein/min)	0.346±0.075	0.522±0.088*	0.416±0.030	0.476±0.068*

Wistar rats were given diets containing curcumin (0.05, 0.5 and 5 g/kg diet) for 4 weeks. Each value is the mean±S.D. for five rats. Significant difference from the untreated control group is indicated by $*P<0.01$.

PLSD. A P-value <0.05 was considered to be significant. These statistical analyses were performed using a computer program (Stat View 5.0, ASA Institute Inc., Cary, NC, USA).

Results

Dose-dependent effects of curcumin on the hepatic CYP activity

During the curcumin treatment, there was no difference in the dietary intake or the body weight gain between each group. The average intake dose of curcumin was calculated based on the intake amount of diet and the body weight of the group, at a dose of 0.05 g/kg diet for about 6.2 mg/kg body weight per day. The effects of curcumin on the weights of body and liver, and hepatic metabolizing enzymes of rats are shown in Table 1. Curcumin had no influence on the liver weight and hepatic CYP content in rats (Table 1). Glutathione *S*-transferase activity increase correlated with the ingestion of curcumin (Table 1). The effects of curcumin on the various CYP activities of rats are shown in Table 2. Treatment with curcumin (0.05 and 0.5 g/kg diet) did not change the activity of the six types of CYP, while the extremely high dose (5 g/kg diet) of curcumin tended to increase the activity of pentoxyresorufin *O*-dealkylase as corresponding to CYP2B and (*S*)-warfarin 7-hydroxylase as CYP2C9 (Table 2).

Effects of curcumin on the changes of hepatic CYP activities induced by chronic CCl_4 injection in rats

Liver weight was increased with CCl_4 treatment, by 1.2-fold (4.32±0.12%/body weight, $P<0.05$) compared with the untreated control group (3.69±0.13%), and slight inductions were observed by co-administration of curcumin. The liver weights were 4.60±0.22% in 0.05 g/kg diet group, 4.59±0.17% in 0.5 g/kg diet group and 4.64±0.21% in 5 g/kg diet group, respectively. The GOT and GPT activities in plasma were significantly increased by chronic CCl_4 treatment: 40.3±4.8 IU/L and 17.6±2.2 IU/L in the untreated control group, 120±7.4 IU/L and 78.9±19.3 IU/L in the CCl_4-treated group. Repeated administrations of curcumin did not influence the increases in GOT and GPT activities, even high dose 0.5 g/kg diet: 105±11.1 IU/L and 87.7±10.9 IU/L, respectively. Effects of curcumin on the changes in the content of CYP and the activities of the CYPs and glutathione *S*-transferase in CCl_4-treated rats are shown in Fig. 2 and Table 3. Chronic CCl_4 treatment markedly decreased hepatic total CYP content to 29%, compared to the level of the untreated control group (Fig. 2A). In contrast, the ingestion of higher doses of curcumin (0.5 and 5 g/kg diet) significantly moderated the reduction of CYP content to 55% of the level of the untreated control group. Similarly, the activities of the six types of CYPs were drastically decreased by CCl_4 treatment, while higher doses of curcumin (0.5 and 5 g/kg diet) inhibited the decreases of CYP activity, except for *p*-nitrophenol hydroxylase corresponding to CYP2E1 (Table 3). Glutathione *S*-transferase activity was reduced by CCl_4 treatment, while the effects of co-administered curcumin were not significant (Fig. 2B).

Discussion

The objectives of the present study were two-fold: firstly, to examine the effects of curcumin on hepatic CYP activity in order to analyze hepatic drug-metabolizing function and CYP-

Table 2
Effects of curcumin on the activity of various hepatic CYPs in rats

	Untreated control	Curcumin		
		(0.05 g/kg)	(0.5 g/kg)	(5 g/kg)
	Activity (pmol/mg protein/min)			
Ethoxyresorufin *O*-deethylase (CYP1A1)	10.1±2.80	10.1±1.22	9.94±1.17	12.7±2.02
Methoxyresorufin *O*-demethylase (CYP1A2)	6.19±1.34	6.41±0.93	5.95±0.92	6.68±1.19
Pentoxyresorufin *O*-dealkylase (CYP2B)	2.71±0.76	2.71±0.36	2.69±0.23	3.58±0.70*
(*S*)-Warfarin 7-hydroxylase (CYP2C9)	2.09±0.61	2.05±0.25	2.13±0.24	3.02±0.52*
p-Nitrophenol hydroxylase (CYP2E1)	7330±1235	7162±674	6531±527	7331±872
Testosterone 6β-hydroxylase (CYP3A)	1641±506	1425±157	1505±115	1693±157

Wistar rats were fed diets containing curcumin (0.05, 0.5 and 5 g/kg diet) for 4 weeks. Each value is the mean±S.D. for five rats. Significant difference from the untreated control group is indicated by $*P<0.05$.

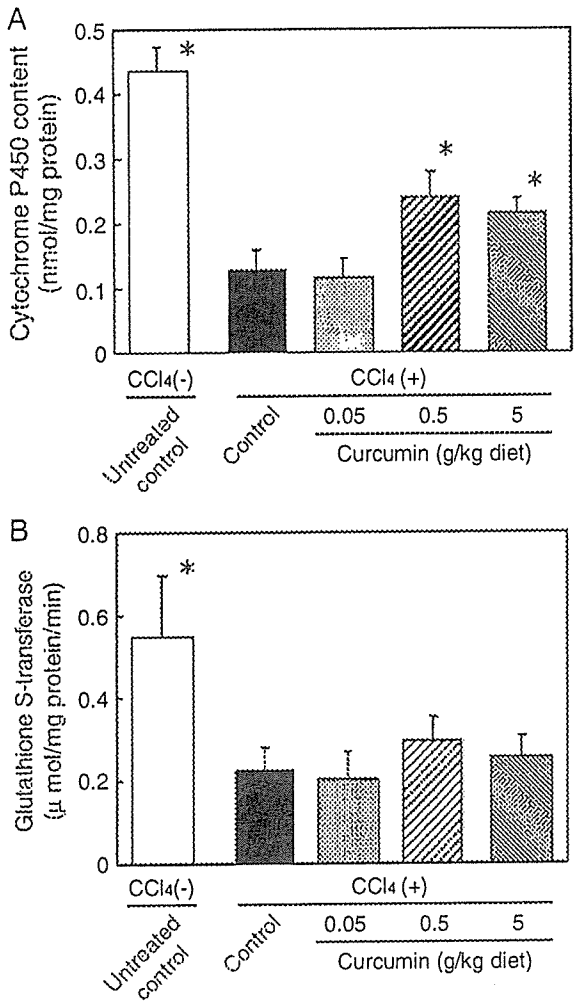

Fig. 2. Effects of curcumin on the reduction of hepatic cytochrome P450 content and glutathione *S*-transferase activity induced by CCl_4 injection. Wistar rats were fed diets containing curcumin (0.05, 0.5 and 5 g/kg diet) for 7 weeks and intraperitoneally injected with CCl_4 twice a week. (A) Cytochrome P450 content, (B) glutathione *S*-transferase activity. Each column is the mean±S.D. for five rats. Significant difference from the CCl_4-treated control group is indicated by $^*P<0.01$.

mediated drug interaction, and secondly, to determine the protective effect of curcumin on hepatic CYP damage induced by chronic CCl_4 injection.

As shown in the results, rats were given curcumin-containing diets for 4 consecutive weeks. The dose of curcumin was calculated to be about 5 mg/kg body weight per day based on the recommended dose of curcumin (500 mg/day or more) in human therapy (Sharma et al., 2004; Cheng et al., 2001), and the 10- and 100-fold doses (50 and 500 mg/kg body weight) were also tested. Even in the highest dose group (5 g/kg in diet; about 500 mg/kg body weight), the repeated ingestion of curcumin had no effect on body weight gain, liver weight or the total content of hepatic CYP enzyme of rats (Table 1). Likewise, the activities of six CYP isozymes remained unchanged after curcumin treatment at doses of 0.05 g/kg diet, i.e. 5 mg/kg body weight (Table 2). These results indicate that daily doses of curcumin have no influence on hepatic CYP activities, namely phase I drug-metabolizing enzymes. On the other hand, curcumin increased the activity of glutathione *S*-transferase, one of phase II drug-metabolizing enzymes (Table 1), as previous reports (Iqbal et al., 2003; Okada et al., 2001). Glutathione *S*-transferase is a soluble protein located in the cytosol, and plays an important role in the detoxification and excretion of xenobiotics (Mannervik, 1985; Mannervik et al., 1985). Compounds that increase the glutathione *S*-transferase activity and convert toxic substances to nontoxic substances are known to protect the liver. Some reports have indicated that curcumin increases intracellular glutathione levels and activities of glutathione *S*-transferase and some antioxidative enzymes (Okada et al., 2001; Rinaldi et al., 2002; Piper et al., 1998; Iqbal et al., 2003). These results indicate that curcumin might be beneficial for glutathione-mediated detoxification of electrophilic products of lipid peroxidation.

Recently, herb–drug interactions have become a concern in clinical therapy. Alternative remedies containing curcumin or turmeric are consumed by many patients receiving medical therapy for liver disease (Luper, 1999; Miquel et al., 2002). The fact that repeated intake of curcumin has no influence on hepatic CYP activity suggests that curcumin does not change the efficacy or pharmacokinetics of co-administered medicines. Moreover, because CYPs mediate the biosynthesis and metabolisms of various hormones, it is unlikely that a daily dose of curcumin cause adverse reactions involved in changes of CYP activity.

The free radical scavenging activity of curcumin is beneficial to liver injury caused by a variety of hepatotoxic substances, including CCl_4, ethanol, pentobarbital and acetaminophen (Luper, 1999; Miquel et al., 2002; Park et al., 2000). However, the changes of various CYP activities are not clear in simultaneous injection of CCl_4 and curcumin in rats. Thus, we focused on the changes in CYP activity in CCl_4-induced hepatopathy-modeled rats. Chronic CCl_4 injection increased the liver weight, while drastically reducing the content and activity of CYP enzymes, especially CYP2E1, as shown by the *p*-nitrophenol hydroxylase activity (Fig. 2A, Table 3). These results support recent reports of the reductions of mRNA expression and activity of some CYP enzymes in the liver of rats given various doses of CCl_4 (Lee et al., 2004). CYP2E1 is the major isozyme involved in CCl_4 bioactivation and generated cytotoxic trichloromethyl radicals are thought to cause hepatotoxicity (Recknagel et al., 1989; Wong et al., 1998; Williams and Burk, 1990). Furthermore, alterations in CYP2E1 activity can affect susceptibility to hepatic injury from CCl_4 (Wong et al., 1998; Takahashi et al., 2002). Moreover, the reactive free radicals inactivate CYP enzymes and subsequent depletion of CYP2E1 (Guengerich et al., 1991; Jeong, 1999; Zhou et al., 2004). In this way, CCl_4 injection decreased the CYPs 1A, 2B, 2C and 3A isozymes activities, similar to CYP2E1 (Table 3). In contrast, repeated curcumin ingestion in higher doses (0.5 and 5 g/kg diet) significantly relieved the CCl_4-caused reductions of total CYP content (Fig. 2A) and the activities, except for CYP2E1

Table 3
Effects of curcumin on the activity of various hepatic CYPs in rats treated with and without CCl_4

	Untreated control	CCl_4-treated			
		Control	Curcumin		
			(0.05 g/kg)	(0.5 g/kg)	(5 g/kg)
	Activity (pmol/mg protein/min)				
Ethoxyresorufin *O*-deethylase (CYP1A1)	16.92±1.78*	3.70±1.15	3.16±0.51	7.67±3.42*	6.67±2.67
Methoxyresorufin *O*-demethylase (CYP1A2)	10.31±0.49*	1.97±0.27	1.65±0.14	3.43±0.49*	3.22±0.47
Pentoxyresorufin *O*-dealkylase (CYP2B)	3.25±0.37*	1.28±0.18	1.24±0.13	1.82±0.36*	1.77±0.49*
(*S*)-Warfarin 7-hydroxylase (CYP2C9)	1.00±0.25*	0.146±0.044	0.192±0.042	0.416±0.142*	0.419±0.276
p-Nitrophenol hydroxylase (CYP2E1)	6444±1043*	462±147	419±127	458±148	462±67
Testosterone 6β-hydroxylase (CYP3A)	917±171*	340±63	224±63	579±263*	502±237

Wistar rats were fed diets containing curcumin (0.05, 0.5 and 5 g/kg diet) for 7 weeks and intraperitoneally injected with CCl_4 twice a week. Each value is the mean±S.D. for five rats. Significant difference from the CCl_4-treated control group is indicated by $*P<0.05$.

(Table 3). Glutathione *S*-transferase activity was also decreased by CCl_4 treatment and co-administered curcumin (0.5 g/kg diet) tended to recover the decreased activity, but there was no significance. The activities of GOT and GPT, well-known biomarkers, were markedly elevated by CCl_4 injection, indicating severe tissue damage. Co-administered curcumin, even in high dose, did not inhibit the increase in these activities. These results suggested that curcumin did not significantly relieve tissue damage by CCl_4 as indicated by the transaminase activities, but relieved the decreased hepatic CYPs activity in the present experimental condition. Interestingly, among the six CYP enzymes examined, CYP2E1 was degraded the most by CCl_4 injection and no amelioration was observed with curcumin ingestion (Table 3). CYP2E1-mediated metabolism of CCl_4 generated reactive free radicals, and CYP2E1 protein might be more susceptible to CCl_4 toxicity than other CYP isozymes. Curcumin could not moderate the decrease of CYP2E1 activity. In other words, curcumin was unavailable to additionally precipitate the bioactivation of CCl_4 and exacerbated liver damage. Curcumin did not change the hepatic CYP activity in normal rats (Tables 1 and 2), indicating that curcumin indirectly improved the inactivation of CYPs induced by severe CCl_4 toxicity.

Many previous investigations regarding CCl_4-induced liver injury have focused only on CYP2E1 activity, but not on other CYP isoforms (Yokogawa et al., 2004; Jeong et al., 2002; Jeon et al., 2003). In this study, different susceptibilities to CCl_4 were observed between in CYP2E1 and other isozymes, i.e. CYPs 1A, 2B, 2C and 3A, and the effects of curcumin were also different. The mechanism underlying the CCl_4-induced degradation of CYP activity may be different between CYP2E1 and other isoforms. CYP2E1 mediated CCl_4 bioactivation and produced reactive free radicals, and accordingly, the most suicidal damaged among the CYP isozymes. It is speculated that the antioxidant properties of curcumin inhibit the secondary inactivation of CYPs caused by reactive free radicals.

In conclusion, curcumin ingestion has no influence on hepatic CYP activity in rats, indicating no pharmacokinetic interaction with co-administered drugs. Curcumin does not prevent the decrease of CYP2E1 activity related to the first step of metabolic activation of CCl_4. However, curcumin is beneficial for ameliorating the subsequent inactivation of other CYP isozymes caused by CCl_4. The antioxidant properties of curcumin may contribute to the inhibition of the reactive free radicals produced from CCl_4 bioactivation. Further detail study will be needed to clarify the mechanism of curcumin against CCl_4-induced liver injury.

References

Ammon, H.P., Wahl, M.A., 1991. Pharmacology of *Curcuma longa*. Planta Medica 57, 1–7.

Asai, A., Miyazawa, T., 2001. Dietary curcuminoids prevent high-fat diet-induced lipid accumulation in rat liver and epididymal adipose tissue. The Journal of Nutrition 131, 2932–2935.

Brattin, W.J., Glende Jr., E.A., Recknagel, R.O., 1985. Pathological mechanisms in carbon tetrachloride hepatotoxicity. Journal of Free Radicals in Biology and Medicine 1, 27–38.

Brautbar, N., Williams, J., 2002. 2nd industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms. International Journal of Hygiene and Environmental Health 205, 479–491.

Cheng, A.L., Hsu, C.H., Lin, J.K., Hsu, M.M., Ho, Y.F., Shen, T.S., Ko, J.Y., Lin, J.T., Lin, B.R., Ming-Shiang, W., Yu, H.S., Jee, S.H., Chen, G.S., Chen, T.M., Chen, C.A., Lai, M.K., Pu, Y.S., Pan, M.H., Wang, Y.J., Tsai, C.C., Hsieh, C.Y., 2001. Phase 1 clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Research 21, 2895–2900.

De Smet, P.A., 2002. Herbal remedies. The New England Journal of Medicine 347, 2046–2056.

Ernst, E., 2002. The risk-benefit profile of commonly used herbal therapies: Ginkgo, St. John's Wort, Ginseng, Echinacea, Saw Palmetto, and Kava. Annals of Internal Medicine 136, 42–53.

Govindarajan, V.S., 1980. Turmeric—chemistry, technology, and quality. Critical Reviews in Food Science and Nutrition 12, 199–301.

Guengerich, F.P., Kim, D.H., Iwasaki, M., 1991. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. Chemical Research in Toxicology 4, 168–179.

Habig, W.H., Jakoby, W.B., 1981. Assays for differentiation of glutathione *S*-transferases. Methods in Enzymology 77, 398–405.

Hanioka, N., Tatarazako, N., Jinno, H., Arizono, K., Ando, M., 2000. Determination of cytochrome P450 1A activities in mammalian liver microsomes by high-performance liquid chromatography with fluorescence detection. Journal of Chromatography. B, Biomedical Sciences and Applications 744, 399–406.

Iqbal, M., Sharma, S.D., Okazaki, Y., Fujisawa, M., Okada, S., 2003. Dietary supplementation of curcumin enhances antioxidant and phase II metabolizing enzymes in ddY male mice: possible role in protection against chemical carcinogenesis and toxicity. Pharmacology and Toxicology 92, 33–38.

Jeon, T.I., Hwang, S.G., Park, N.G., Jung, Y.R., Shin, S.I., Choi, S.D., Park, D.K., 2003. Antioxidative effect of chitosan on chronic carbon tetrachloride induced hepatic injury in rats. Toxicology 187, 67–73.

Jeong, H.G., 1999. Inhibition of cytochrome P450 2E1 expression by oleanolic acid: hepatoprotective effects against carbon tetrachloride-induced hepatic injury. Toxicology Letters 105, 215–222.

Jeong, H.G., You, H.J., Park, S.J., Moon, A.R., Chung, Y.C., Kang, S.K., Chun, H.K., 2002. Hepatoprotective effects of 18beta-glycyrrhetinic acid on carbon tetrachloride-induced liver injury: inhibition of cytochrome P450 2E1 expression. Pharmacological Research 46, 221–227.

Khopde, S.M., Priyadarsini, K.I., Guha, S.N., Satav, J.G., Venkatesan, P., Rao, M.N., 2000. Inhibition of radiation-induced lipid peroxidation by tetrahydrocurcumin: possible mechanisms by pulse radiolysis. Bioscience, Biotechnology, and Biochemistry 64, 503–509.

Kubota, Y., Kobayashi, K., Tanaka, N., Nakamura, K., Kunitomo, M., Umegaki, K., Shinozuka, K., 2004. Pretreatment with *Ginkgo biloba* extract weakens the hypnosis action of phenobarbital and its plasma concentration in rats. The Journal of Pharmacy and Pharmacology 56, 401–405.

Lang, D., Bocker, R., 1995. Highly sensitive and specific high-performance liquid chromatographic analysis of 7-hydroxywarfarin, a marker for human cytochrome P-4502C9 activity. Journal of Chromatography. B, Biomedical Applications 672, 305–309.

Lee, K.J., Woo, E.R., Choi, C.Y., Shin, D.W., Lee, D.G., You, H.J., Jeong, H.G., 2004. Protective effect of acteoside on carbon tetrachloride-induced hepatotoxicity. Life Sciences 74, 1051–1064.

Luper, S., 1999. A review of plants used in the treatment of liver disease: part two. Alternative Medicine Review 4, 178–188.

Mannervik, B., 1985. The isoenzymes of glutathione transferase. Advances in Enzymology and Related Areas of Molecular Biology 57, 357–417.

Mannervik, B., Alin, P., Guthenberg, C., Jensson, H., Tahir, M.K., Warholm, M., Jornvall, H., 1985. Identification of three classes of cytosolic glutathione transferase common to several mammalian species: correlation between structural data and enzymatic properties. Proceedings of the National Academy of Sciences of the United States of America 82, 7202–7206.

Miquel, J., Bernd, A., Sempere, J.M., Diaz-Alperi, J., Ramirez, A., 2002. The curcuma antioxidants: pharmacological effects and prospects for future clinical use. A review. Archives of Gerontology and Geriatrics 34, 37–46.

Mishin, V.M., Koivisto, T., Lieber, C.S., 1996. The determination of cytochrome P450 2E1-dependent *p*-nitrophenol hydroxylation by high-performance liquid chromatography with electrochemical detection. Analytical Biochemistry 233, 212–215.

Nanji, A.A., Jokelainen, K., Tipoe, G.L., Rahemtulla, A., Thomas, P., Dannenberg, A.J., 2003. Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of NF-kappa B-dependent genes. American Journal of Physiology: Gastrointestinal and Liver Physiology 284, G321–G327.

Okada, K., Wangpoengtrakul, C., Tanaka, T., Toyokuni, S., Uchida, K., Osawa, T., 2001. Curcumin and especially tetrahydrocurcumin ameliorate oxidative stress-induced renal injury in mice. The Journal of Nutrition 131, 2090–2095.

Omura, T., Sato, R., 1964. The carbon monoxide-binding pigment of liver microsomes: I. Evidence for its hemoprotein nature. The Journal of Biological Chemistry 239, 2370–2378.

Park, E.J., Jeon, C.H., Ko, G., Kim, J., Sohn, D.H., 2000. Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. The Journal of Pharmacy and Pharmacology 52, 437–440.

Piper, J.T., Singhal, S.S., Salameh, M.S., Torman, R.T., Awasthi, Y.C., Awasthi, S., 1998. Mechanisms of anticarcinogenic properties of curcumin: the effect of curcumin on glutathione linked detoxification enzymes in rat liver. The International Journal of Biochemistry and Cell Biology 30, 445–456.

Ramirez-Tortosa, M.C., Mesa, M.D., Aguilera, M.C., Quiles, J.L., Baro, L., Ramirez-Tortosa, C.L., Martinez-Victoria, E., Gil, A., 1999. Oral administration of a turmeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis. Atherosclerosis 147, 371–378.

Recknagel, R.O., Glende Jr., E.A., Dolak, J.A., Waller, R.L., 1989. Mechanisms of carbon tetrachloride toxicity. Pharmacology and Therapeutics 43, 139–154.

Reeves, P.G., Nielsen, F.H., Fahey Jr., G.C., 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. The Journal of Nutrition 123, 1939–1951.

Rinaldi, A.L., Morse, M.A., Fields, H.W., Rothas, D.A., Pei, P., Rodrigo, K.A., Renner, R.J., Mallery, S.R., 2002. Curcumin activates the aryl hydrocarbon receptor yet significantly inhibits (−)-benzo(*a*)pyrene-7*R*-*trans*-7,8-dihydrodiol bioactivation in oral squamous cell carcinoma cells and oral mucosa. Cancer Research 62, 5451–5456.

Rukkumani, R., Aruna, K., Varma, P.S., Rajasekaran, K.N., Menon, V.P., 2004. Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. Journal of Pharmacy and Pharmaceutical Sciences 7, 274–283.

Sharma, R.A., Euden, S.A., Platton, S.L., Cooke, D.N., Shafayat, A., Hewitt, H.R., Marczylo, T.H., Morgan, B., Hemingway, D., Plummer, S.M., Pirmohamed, M., Gescher, A.J., Steward, W.P., 2004. Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. Clinical Cancer Research 10, 6847–6854.

Sugiyama, T., Kubota, Y., Shinozuka, K., Yamada, S., Wu, J., Umegaki, K., 2004. *Ginkgo biloba* extract modifies hypoglycemic action of tolbutamide via hepatic cytochrome P450 mediated mechanism in aged rats. Life Sciences 75, 1113–1122.

Takahashi, S., Takahashi, T., Mizobuchi, S., Matsumi, M., Morita, K., Miyazaki, M., Namba, M., Akagi, R., Hirakawa, M., 2002. Increased cytotoxicity of carbon tetrachloride in a human hepatoma cell line overexpressing cytochrome P450 2E1. The Journal of International Medical Research 30, 400–405.

Umegaki, K., Saito, K., Kubota, Y., Sanada, H., Yamada, K., Shinozuka, K., 2002. *Ginkgo biloba* extract markedly induces pentoxyresorufin *O*-dealkylase activity in rats. Japanese Journal of Pharmacology 90, 345–351.

Williams, A.T., Burk, R.F., 1990. Carbon tetrachloride hepatotoxicity: an example of free radical-mediated injury. Seminars in Liver Disease 10, 279–284.

Williamson, E.M., 2001. Synergy and other interactions in phytomedicines. Phytomedicine 8, 401–409.

Wong, F.W., Chan, W.Y., Lee, S.S., 1998. Resistance to carbon tetrachloride-induced hepatotoxicity in mice which lack CYP2E1 expression. Toxicology and Applied Pharmacology 153, 109–118.

Yokogawa, K., Watanabe, M., Takeshita, H., Nomura, M., Mano, Y., Miyamoto, K., 2004. Serum aminotransferase activity as a predictor of clearance of drugs metabolized by CYP isoforms in rats with acute hepatic failure induced by carbon tetrachloride. International Journal of Pharmaceutics 269, 479–489.

Zhou, S., Koh, H.L., Gao, Y., Gong, Z.Y., Lee, E.J., 2004. Herbal bioactivation: the good, the bad and the ugly. Life Sciences 74, 935–968.

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Relationship between *Garcinia cambogia*-Induced Impairment of Spermatogenesis and Meiosis-Activating Sterol Production in Rat Testis

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Summary **Dietary supplements for body fat reduction have become popular, particularly in developed countries. *Garcinia cambogia* (GA) is one such supplement, and its active component is (–)-hydroxycitric acid ((–)-HCA), a competitive inhibitor of ATP citrate lyase, which is responsible for producing acetyl CoA from citric acid. Recently we have found that administration of (–)-HCA-containing GA markedly reduces testis weight in male Zucker obese rats. In particular, histopathological examinations revealed testicular atrophy and impairment of spermatogenesis. In the present study, we investigated the cause of the impaired spermatogenesis after ingestion of GA containing (–)-HCA at 102 mmol/kg diet in young Fischer 344 male rats. Among hormones related to spermatogenesis, the serum level of inhibin-B was significantly lower and that of follicle-stimulating hormone (FSH) was higher in the GA group. The level of testis meiosis-activating sterol (T-MAS), which is an intermediate in cholesterol biosynthesis from acetyl CoA and is presumed to transmit a signal for spermatogenesis, was statistically lower in the testes of rats administered GA. We hypothesize from these results that (–)-HCA-mediated inhibition of ATP citrate lyase in rats fed GA leads to diminished accumulation of MAS substances, thus resulting in impairment of spermatogenesis.**

Key Words: ***Garcinia cambogia*, (–)-hydroxycitric acid, testis-meiosis activating sterol, inhibin-B, spermatogenesis**

Introduction

Dietary supplements for body fat reduction have become popular, particularly in developed countries. One ingredient of such dietary supplements is an extract of *Garcinia cambogia* (GA), a fruit grown in Southeast Asia and India. The rind of GA contains hydroxycitric acid (HCA), and four isomers of HCA with their free and lactone forms are found in the extract [*1*]. Among them, only (–)-HCA is a potent competitive inhibitor of ATP citrate lyase (EC 4.1.3.8) [*2*].

Citric acid, produced by glycolysis and then transported into the cytosol from mitochondria, is an important substrate for ATP citrate lyase, which converts citric acid to acetyl CoA and oxaloacetic acid. Hence, ATP citrate lyase is a key enzyme in the supply of acetyl CoA for both *de novo* fatty acid and cholesterol biosyntheses. Lowenstein [*3*] determined the effect of (–)-HCA on fatty acid biosynthesis in rat liver by measuring the incorporation of ^{3}H from $^{3}H_2O$ and showed that fatty acid biosynthesis was inhibited strongly by (–)-HCA. Sullivan *et al.* [*4*] observed the effect of isomers of HCA on lipogenesis in rat liver by using [^{14}C]citrate and [^{14}C]alanine, and obtained similar results.

Recently, our group [*5*] examined the effect of GA

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administration on body fat accumulation in male Zucker obese rats. The rats were fed diets containing GA powder S® ((−)-HCA levels ; 0, 10, 51, 102 and 154 mmol/kg diet) for 92 or 93 days. Surprisingly, the high doses of (−)-HCA-containing GA (102 mmol/kg diet or higher) caused testicular atrophy and impairment of spermatogenesis. From the results, a diet containing 51 mmol/kg was considered to be the no observed adverse effect level (NOAEL) in these rats.

It has been shown recently that 4,4-dimethyl-5α-cholesta-8,24-diene-3β-ol (testis meiosis-activating sterol; T-MAS) is a specific intermediate product of cholesterol biosynthesis in testicular germ cells [*6*]. T-MAS was isolated and characterized from bull testes [*7*]. Similarly, 4,4-dimethyl-5α-cholesta 8,14,24-triene-3β-ol (follicular fluid meiosis-activating sterol; FF-MAS) was isolated from human follicular fluid [*7*]. These MAS substances are produced from lanosterol by the action of lanosterol 14α-demethylase (CYP51) and sterol Δ14-reductase in the cholesterol biosynthetic pathway (Fig. 1). Interestingly, FF-MAS and T-MAS are presumed to be signaling substances that trigger the start of meiotic division of the oocyte and spermatocyte, respectively [*8*]. Indeed, Grondahl *et al.* have demonstrated that FF-MAS has the ability to reinitiate meiosis in a mouse oocyte assay *in vitro* [*9*].

Therefore, in the present study, we examined the relationship between impaired spermatogenesis and MAS substances production in rat testis after administration of (−)-HCA-containing GA.

Materials and Methods

Materials

Garcinia cambogia powder S® was generously donated by Nippon Shinyaku Co.Ltd., Japan. The (−)-HCA content of this powder was 41.2wt% and the ratio of its free to lactone form was 36.6 to 63.4.

Animals

This experiment was carried out under the guidelines of the Animal Committee of Incorporated Administrative Agency, National Institute of Health and Nutrition (Tokyo, Japan).

Three-week-old male Fischer 344/DuCrj rats were purchased from Charles River Japan, Inc. (Yokohama, Japan). They were kept individually in stainless steel cages at 22 ± 1°C and 50–60% humidity with a 12 h light/dark cycle. The feed and water were supplied *ad libitum*. The composition of diets based on the AIN-93G purified diet for

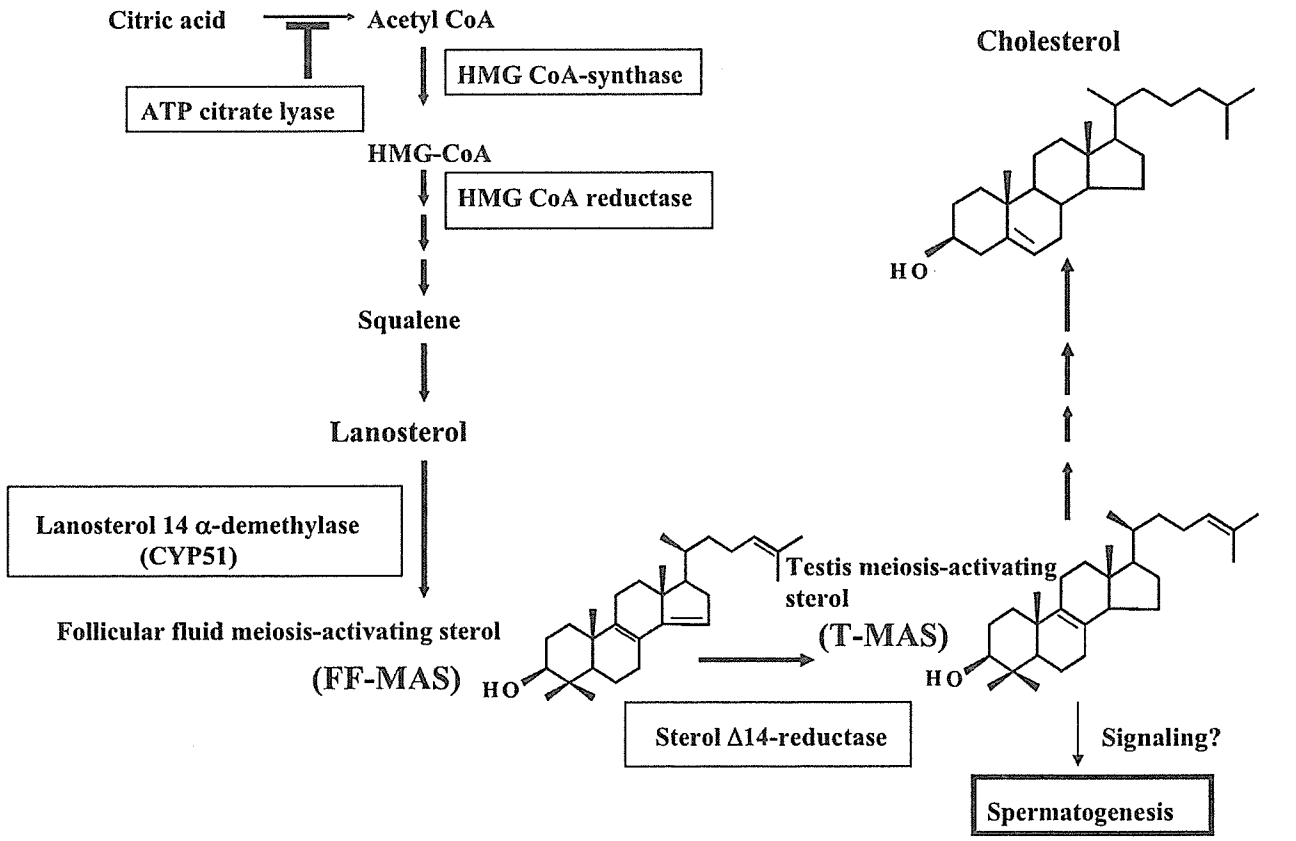

Fig. 1. Proposed biosynthetic pathway of follicular fluid meiosis-activating sterol (FF-MAS), testis meiosis-activating sterol (T-MAS) and cholesterol in rat testis.