＜Adverse Events Causality Evaluation Scale for Health Food＞
本評価はあくまでもスクリーニング目的で使用するものです。

有害事象を評価するために以下の質問に答え、適切な点数をつけてください

No.	質問項目	はい	いいえ[*1]	不詳
1.	生じた有害事象は、当該健康食品の添付文書やラベルに記載されているものですか？	+1	0	0
2.	当該健康食品を摂取した後に、有害事象が現れましたか？	+2	-1	0
3.	当該健康食品を中止した際、有害事象は改善されましたか？	+2	0	0
4.	当該健康食品を再摂取した際、有害事象はまた現れましたか？	+3	-1	0
5.	その有害事象を引き起こすかもしれない（当該健康食品以外の）他の要因[*2]はありますか？	-1	+2	0
6.	その有害事象は摂取量を増量したとき程度は重くなり、減量したとき軽くなりましたか？	+1	0	0
7.	以前に、同じかあるいは類似の健康食品または医薬品で同様の有害事象が現れましたか？	+1	0	0
8.	その有害事象は客観的証拠[*3]によって確かめられましたか？	+2	0	0

合計点 [   ]

「いいえ」という答えは、どのような代替案を考慮したとしても、十分な情報が存在しない場合を前提とします（不確かなとき、あるいは情報不足で評価できない場合は、「わからない」としてください）。

他の要因としては、基礎疾患や合併症の病態、併用薬や他の健康食品の摂取などを考慮します。

客観的証拠とは、当該健康食品に含まれる成分に対して DLST、パッチテストなどの特異的な検査によって確認されたものです。

合計点による評価判定

9 ≦	非常に確からしい	Highly probable
5−8	確からしい	Probable
3−4	可能性がより強くある	Highly possible
1−2	可能性がある	Possible
≦0	関連なし	Unlikely

注：「質問2」が不詳の場合は、情報不足（Lack of Information）とする。

参考文献:
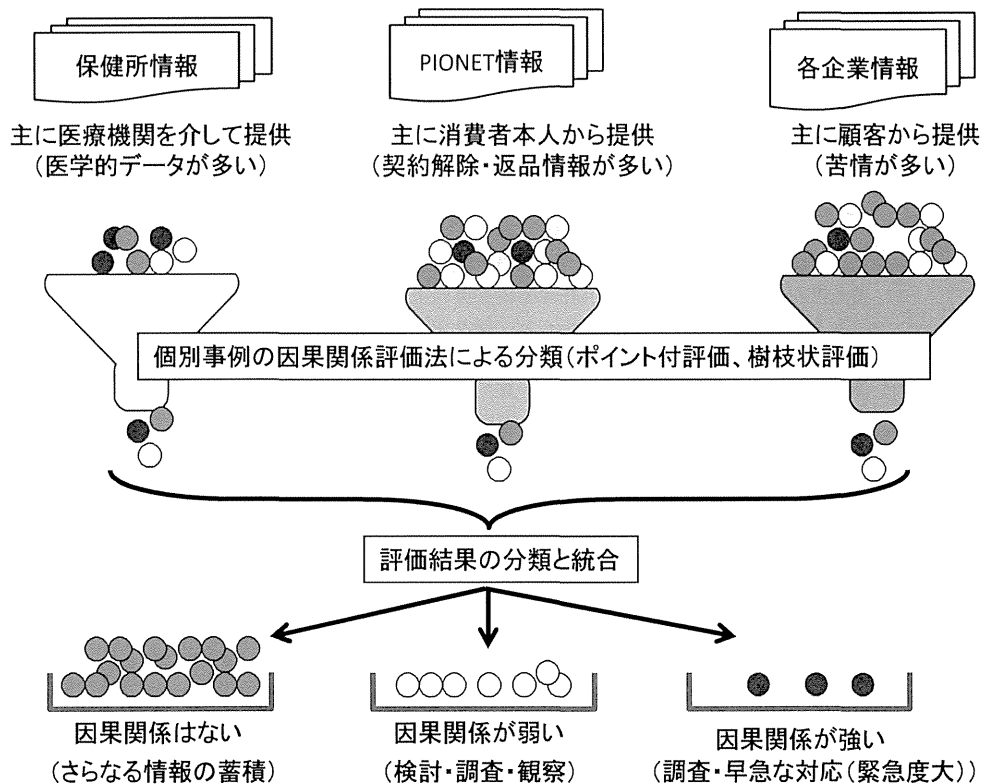松本　圭司ほか. 臨床薬理. 2011；42(4)：211-214.

図 1a　健康食品による健康被害の因果関係評価法（スクリーニング法）

＜Adverse Events Causality Assessment Tool for Health Food＞[1)]

本評価はあくまでもスクリーニング目的で使用するものです。

Ⅰ： ここから開始して評価してください。
　　（□のチェックボックスにレ点を入れてください。）

- その有害事象は、健康食品の摂取後に起こりましたか？
  - 不詳 → □ *Lack of information* 情報不足・評価不能
  - いいえ → □ *Unlikely* 関連なし
  - はい →
- 摂取中止・減量により観察された有害事象は和らぎましたか？
  - いいえ → その有害事象は、他の要因[*1]の影響が考えられますか？
    - はい → □ *Unlikely* 関連なし
    - いいえ・不詳 → その有害事象は客観的証拠[*2]によって確かめられましたか？
      - いいえ・不詳 → □ *Possible* 可能性がある
      - はい → □ *Highly possible* 可能性がより強くある
  - 中止・減量をしていない・不詳 → 以前に同じかあるいは類似の健康食品または医薬品で同様の有害事象があらわれましたか？
    - いいえ → □ *Highly possible* 可能性がより強くある
    - はい・不詳 → その有害事象は客観的証拠[*2]によって確かめられましたか？
      - はい → □ *Highly possible* 可能性がより強くある
      - いいえ・不詳 → その有害事象は、他の要因[*1]の影響が考えられますか？
        - はい・不詳 → □ *Probable* 確からしい
        - いいえ → □ *Probable* 確からしい
  - はい →
- その有害事象は再摂取で再現しましたか？
  - いいえ・再摂取をしていない・不詳 → （以前に同じかあるいは類似の健康食品…へ）
  - はい →
- その有害事象は客観的証拠[*2]によって確かめられましたか？
  - いいえ・不詳 → □ *Probable* 確からしい
  - はい → □ *Highly probable* 非常に確からしい

*1 他の要因としては、基礎疾患や合併症の病態、併用薬やほかの健康食品の摂取などを考慮します。
*2 客観的証拠とは、当該健康食品に含まれる成分に関してDLST、パッチテストなどの特異的な検査によって確認されたものです。

Ⅱ： 健康被害の重篤度[2)]をチェックしてください。

- □ 軽微な健康被害と考えられるもの
- □ 軽度な健康被害と考えられるもの（例：医療機関で治療を要した。）
- □ 中等度の健康被害と考えられるもの（重篤ではないが軽度でもない。）
  　（例：30日以上の治療、または入院・入院の延長を要するものなど）
- □ （死亡・後遺症を残すなど）重篤な健康被害と考えられるもの

参考文献：
1) 山田　浩ほか. 臨床薬理. 2012 ; 43(6) : 399-402.
2) 副作用の重篤度分類基準. 厚生労働省課長通知　平成4年6月29日薬安第80号

図 1b　健康食品による健康被害の因果関係評価法（スクリーニング法）

健康食品に関する有害事象の聞き取り票 （追加様式1）

該当箇所に〇印または空欄に記入してください。従来の「受付票」にもある項目で、重複するときはこの票に記入する必要はありません。この票は必要な情報項目を示したもので詳しい情報の収集を制限するものではありません。

ID		聞き取り日　　　2013 年　　　　月　　　　日		
報告者（該当に〇）		本人　　　家族　　　医療関係者　　　その他（　　　　　　　）		
**** 以下は有害事象を受けた人に関する情報　****				
1	性別（該当に〇）	男	女	不詳
2	年齢	歳代		不詳
3	居住地	郵便番号、住所、固定電話の市外局番のいずれか		不詳
4	製品名			
5	症状 （該当に〇 または記入）	発疹・発赤・掻痒 吐き気・嘔吐　　胃痛・腹痛　　　下痢　　便秘 肝機能障害（肝機能検査値の上昇） 腎機能障害（むくみ、尿タンパクの出現） 呼吸器障害（息切れ、呼吸困難） 循環器障害（血圧上昇、動悸・頻脈） 神経障害（頭痛、めまい） 血液障害（貧血症状、出血症状） その他（　　　　　　　　　　　　　　　　　　　）		
6	症状の発生時期	年　　　月　　　日		不詳
7	医療機関受診 （該当に〇または 記入）	無 有　（医学的検査：　無　　　　有　　　　　　　　）		不詳
8	転帰（該当に〇）	自然治癒　　外来で治療　　入院　　後遺症　　　死亡 （　　　年　　　月　　　日）		不詳
9	製品の摂取状況 （該当箇所に記入） （該当に〇）	期間　　　年　月　日　から　　　　年　月　日　まで 摂取量　　　　　　　　　錠・カプセル・包（粉末） 摂取頻度　　　　　　　　回　日 / 週 減量・中止の有無：　　無　　　　有 （減量・中止後の症状：　改善　　変化なし　不詳　） 再摂取の有無：　　無　　　　有		不詳 不詳 不詳 不詳 不詳
10	他の 健康食品・医薬品の 併用状況 （該当に〇 または記入）	健康食品　　　無 　　　　　　　　有（製品名：　　　　　　　　　　　） 医薬品　　　無 　　　　　　　有（製品名：　　　　　　　　　　　）		不詳
11	基礎疾患・体質 （該当に〇 または記入）	基礎疾患：　　　無　　　　有 　　　　　　　（疾患名：　　　　　　　　　　　　） アレルギー体質：　無　　　有 　　　　（食物・医薬品・花粉・ハウスダスト）		不詳
相談者からの返品の要求（参考情報）　（該当に〇）		無　　　　　　　　有		不詳

図2　健康食品による健康被害情報の聞き取り票

保健所情報

主に医療機関を介して提供
（医学的データが多い）

PIONET情報

主に消費者本人から提供
（契約解除・返品情報が多い）

各企業情報

主に顧客から提供
（苦情が多い）

個別事例の因果関係評価法による分類（ポイント付評価、樹枝状評価）

評価結果の分類と統合

因果関係はない
（さらなる情報の蓄積）

因果関係が弱い
（検討・調査・観察）

因果関係が強い
（調査・早急な対応（緊急度大））

図3　異なる機関・組織で収集された情報の分析・整理による行政的な対応の概念図

別紙　研究成果の刊行に関する一覧表

雑誌

発表者名	論文タイトル名	発表誌名	巻	ページ	出版年
山田浩、一丸佳代、小野彩奈、高橋光明、松本圭司、小菅和仁、藤本和子、脇昌子、中島光好、梅垣敬三.	健康食品の摂取に伴う有害事象の因果関係評価のための樹枝状アルゴリズムの構築.	臨床薬理	43	399-402	2012
Yokotani K, Chiba T, Sato Y, Taki Y, Yamada S, Shinozuka K, Murata M, Umegaki K.	Hepatic cytochrome P450 mediates interaction between warfarin and Coleus forskohlii extract in vivo and in vitro.	J Pharm Pharmacol.	64	1793-1801	2012
横谷馨倫、千葉剛、佐藤陽子、窪田洋子、渡邊康雄、村田容常、梅垣敬三.	Coleus forskohlii エキス中の肝シトクローム P450 誘導物質の推定.	応用薬理	82	67-73	2012
一丸佳代、井出和希、小野彩奈、北川護、成島大智、松本圭司、梅垣敬三、山田浩.	健康食品の摂取に伴う有害事象の因果関係評価のための樹枝状アルゴリズムの改変.	臨床薬理	44	405-410	2013
Virgona N, Taki Y, Yamada S, Umegaki K.	Dietary Coleus forskohlii extract generates dose-related hepatotoxicity in mice.	J Appl Toxicol.	33	924-932	2013
佐藤陽子、中西朋子、横谷馨倫、千葉剛、梅垣敬三.	葉酸およびそのサプリメント摂取に対する妊婦，管理栄養士・栄養士，管理栄養士・看護師養成校の学生の認識.	栄養学雑誌	71	204-212	2013
Yokotani K, Chiba T, Sato Y, Nakanishi T, Murata M, Umegaki K.	Influence of dietary macronutrients on induction of hepatic drug metabolizing enzymes by Coleus forskohlii extract in mice.	J Nutr Sci Vitaminol.	59	37-44	2013

Sato Y, Nakanishi T, <u>Chiba T</u>, Yokotani K, Ishinaga K, Takimoto H, Itoh H, <u>Umegaki K</u>.	Prevalence of inappropriate dietary supplement use among pregnant women in Japan.	Asia Pac J Clin Nutr.	22	83-89	2013
<u>梅垣敬三</u>、山田浩、千葉剛、佐藤陽子、中西朋子、福山哲.	健康食品に関する健康被客事例の情報源およびその有用性評価.	食品衛生学雑誌	54	282-289	2013
北川護、井出和希、成島大智、山田浩.	健康食品の安全性をめぐる最近の話題.	Functional Food	8	52-57	2014
横谷馨倫、<u>千葉剛</u>、佐藤陽子、<u>梅垣敬三</u>.	Coleus forshohlii Extract Attenuates the Hypoglycemic Effect of Tolbutamide in vivo via a Hepatic Cytochrome P450-Mediated Mechanism.	食品衛生学雑誌	55	73-78	2014
<u>千葉剛</u>、佐藤陽子、中西朋子、横谷馨倫、狩野照誉、鈴木祥菜、<u>梅垣敬三</u>.	特定保健用食品の利用実態調査.	日本栄養・食糧学会誌	67	177-184	2014
佐藤陽子、中西朋子、<u>千葉剛</u>、<u>梅垣敬三</u>.	妊婦における神経管閉鎖障害リスク低減のための folic acid 摂取行動に関する全国インターネット調査.	日本公衆衛生雑誌	61	321-332	2014
<u>Umegaki K</u>, Yamazaki Y, Yokotani K, <u>Chiba T</u>, Sato Y, <u>Shimura F</u>.	Induction of fatty liver by Coleus forskohlii extract throughenhancement of de novo triglyceride synthesis in mice.	Toxicology Reports	1	787-794	2014
<u>Chiba T</u>, Sato Y, Nakanishi T, Yokotani Y, Suzuki S, <u>Umegaki K</u>.	Inappropriate usage of dietary supplements in patients by miscommunication with physicians in Japan.	Nutrients.	6	5392-5404	2014

短　報

健康食品の摂取に伴う有害事象の因果関係評価のための
樹枝状アルゴリズムの構築

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中　島　光　好[*4]　　梅　垣　敬　三[*5]

Application of a Dendritic Algorithm for the Evaluation of
Causal Relationships of Adverse Events with Health Food

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緒　論

　近年，健康志向の高まりからサプリメント等の栄養補助食品や，いわゆる健康食品（以下，健康食品と略す）の需要が増加し，それとともに健康食品の摂取に伴う健康被害事例が報告されるようになってきている[1]．健康食品の摂取に伴う健康被害の報告は，ドラッグストアや製造販売元への利用者からの問い合わせ，あるいは医療機関で治療を受けた場合の診療記録等の情報を基に，保健所を介して厚生労働省に集約されていく．また，消費者庁を介しての被害情報の集積も行われている．しかし，これらの情報は種々雑多であり，正確に因果関係評価を行うことは極めて難しく，また科学的に吟味するための臨床上有用な方法論も十分には確立していない．

　すでに我々は，健康食品と医薬品が共に機能的に生体に作用するという類似性に着目し，医薬品の投与に伴う有害事象の因果関係評価において種々開発されて

いる評価法[2]のうち，比較的汎用性の高いアルゴリズムを改変することで，健康食品の摂取に伴う有害事象の因果関係評価法の構築を試みてきた[3~5]．その過程で，まず始めに，評価票形式で質問項目ごとの点数の重み付けを行い加算するNaranjoらの評価票[6]を基にして，健康食品の特性を考慮した改変を重ねてきた[4]．本研究ではNaranjoらの評価票と並び，医薬品の有害事象の因果関係評価で汎用されているJonesの樹枝状アルゴリズム[7]の内容を再検討し，質問項目や分枝形式ならびにカテゴリー分類を改変することで，健康食品に適した，より臨床応用可能な樹枝状アルゴリズムの構築を試みた．

方　法

　すでに作成したJonesの樹枝状アルゴリズムの改変[3]を，健康食品の有する情報の特性を考慮して再検討し，健康食品の摂取に伴い生じた有害事象の因果関係判定に重要と考えられる質問項目や分枝方式ならび

ここから開始して評価してください。
（□のチェックボックスにレ点を入れてください。）

その有害事象は、健康食品の摂取後に起こりましたか？ — 不詳 → □ *Lack of information* 情報不足・評価不能

その有害事象は、他の要因[*1]に起因する可能性が強いですか？ — はい → □ *Remote, Doubtful* （ほぼ関連なし）

摂取中止・減量により観察された有害事象は和らぎましたか？ — いいえ

中止・減量をしていない、不詳 → □ *Possible* （可能性がある）

いいえ・不詳

その有害事象は再摂取で再現しましたか？ — いいえ・再摂取をしていない・不詳

以前に同じかあるいは類似の健康食品または医薬品で同様の有害事象があらわれましたか？ — いいえ → □ *Highly possible* （可能性がより強くある）

その有害事象は、他の要因[*1]に起因するかもしれませんか？ — はい・不詳

いいえ

いいえ・不詳

その有害事象は客観的証拠に[*2]よって確かめられましたか？ — はい → □ *Highly probable, Definite* （非常に確からしい）

□ *Probable* （確からしい）
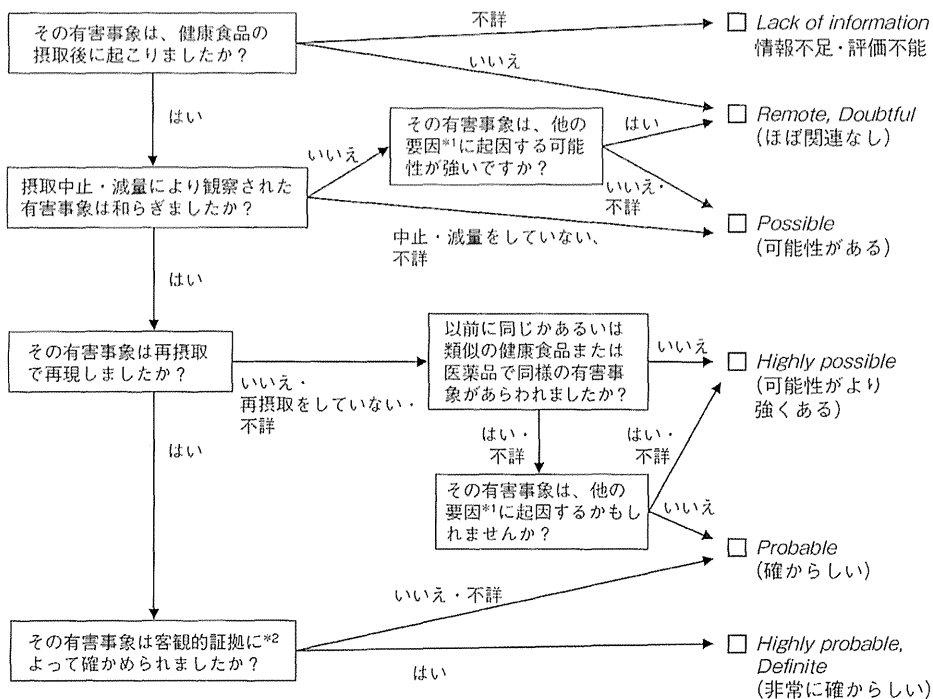
[*1] 他の要因としては、基礎疾患や合併症の病態、併用薬やほかの健康食品の摂取、年齢などを考慮します。
[*2] 客観的証拠とは、当該健康食品に含まれる成分に関してDLST、パッチテストなどの特異的な検査によって確認されたものです。

Fig. 1　健康食品の摂取に伴う有害事象の因果関係評価のために開発した改変樹枝状アルゴリズム

にカテゴリー分類を吟味し，改変を加えた（Fig.1）．改変は，以下の8項目について行った（変更点1〜5は質問項目，6は分枝方式，7〜8はカテゴリー分類における変更点である）．

1）評価開始時の質問項目において，「時間との関連」という表現は曖昧であるため，「摂取後に起こりましたか？」と前後関係を明確にした．

2）摂取中止による症状の変動を問う質問項目において，「摂取中止・減量」に変更することで，減量による変動も評価に加えた．

3）再摂取後の症状の出現を問う質問項目において，症状の再現が見られなかった場合や再摂取をしていない場合には，次に進む質問項目として，「以前に同じかあるいは類似の健康食品または医薬品で同様の有害事象があらわれましたか？」を追加した．

4）再摂取後に症状の再現があった場合，次に進む質問項目として，客観的な検査の有無を問う項目を追加し，注釈「客観的証拠とは，当該健康食品に含まれる成分に関してDLST，パッチテストなどの特異的な検査によって確認されたものです。」を加えた．

5）他の要因を問う質問項目を追加・修正し，「既存の臨床症状」という表現は曖昧であるため，「他の要因」という表現に変え，注釈「他の要因としては，基礎疾患や合併症の病態，併用薬やほかの健康食品の摂取，年齢などを考慮します。」を加えた．

6）分枝方式を，「はい」，「いいえ」の2分枝のみから，「不詳」を加え3つに細分化した．

7）時間的な関連性が不詳の事例は，情報不足「lack of information」のカテゴリー分類とした．

8）すでに作成したNaranjoら調査票の改変によるカテゴリー分類[4]と同様，可能性がある「possible」を，因果関係が強い順に，可能性がより強くある「highly possible」，可能性がある「possible」の2つに細分化した．

次いで今回，改変を加えた樹枝状アルゴリズム（以下，改変樹枝状アルゴリズムと略す）およびNaranjoら評価票の改変を重ねた改変評価票[4]（以下，改変評価票と略す）を用い，健康食品販売業者のお客様センターに寄せられた保健機能食品（特定保健用食品，栄
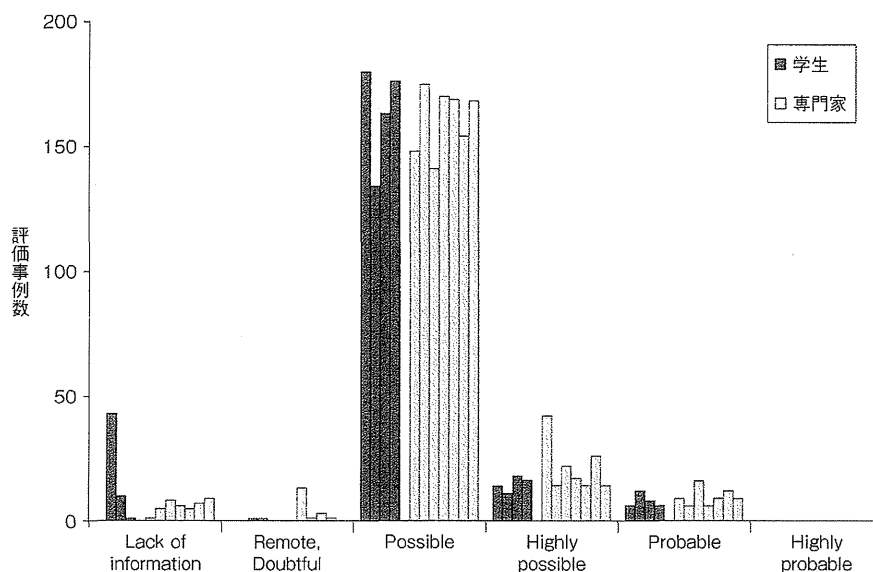
Fig. 2　改変樹枝状アルゴリズムにおける学生および専門家の評価結果の分布
評価者 11 名（薬学系学生 4 名：黒色地，専門家 7 名：灰色地），評価した有害事象事例 200 例

養機能食品）および保健機能食品以外のいわゆる健康食品の摂取に伴う健康被害相談事例 200 例に対して，薬学系学生 4 名および専門家 7 名（医師 3 名，健康食品関連の情報を扱っている専門家 3 名，大学薬学部教員 1 名），計 11 名の評価者により，それぞれ独立に因果関係を評価した．改変樹枝状アルゴリズムにおける評価判定は，因果関係が強い順に，非常に確からしい（highly probable, definite），確からしい（probable），可能性がより強くある（highly possible），可能性がある（possible），ほぼ関連なし（remote, doubtful），情報不足・評価不能（lack of information）の 6 段階にカテゴリー分類した．改変評価票に関しては合計点をスコア化し，改変樹枝状アルゴリズムと同様にカテゴリー分類した．次いで，多評価者間 κ 係数を Fleiss の方法により算出し，両評価法の信頼性を評価した．統計解析は，R ver. 2.15.1（R Development Core Team, 2012）を用いて行った．なお，本研究で利用した健康被害相談事例の個別内容については，機微情報を含むことから提示しないこととした．

結　　果

改変樹枝状アルゴリズムによる 200 事例の評価は，薬学系学生および専門家共に，possible に多く集中し，highly probable はなかった（Fig. 2）．薬学系学生および専門家における改変樹枝状アルゴリズムによる多評価者間 κ 係数は，それぞれ 0.50 と 0.52 であった．一方，薬学系学生および専門家における改変評価票によ

る多評価者間 κ 係数は，それぞれ 0.21 と 0.44 であった．

考　　察

今回の研究では，医薬品の有害事象の因果関係評価に汎用されている Jones の樹枝状アルゴリズムに改変を重ねることで，健康食品の摂取に伴う有害事象の因果関係評価法の構築を試みた．改変樹枝状アルゴリズムと，その基となった Jones の樹枝状アルゴリズムの最大の相違は，健康食品においては有害事象評価に必要な情報が非常に少ないという状況を考慮した点にある．実際，健康食品から得られる情報は医薬品と比べ不十分で，判断が難しいことが多い[1]．この点を考慮し改変樹枝状アルゴリズムでは，選択肢に「はい」，「いいえ」のみでなく「不詳」を加え，カテゴリー分類に，情報不足・評価不能（lack of information）を加えた．さらに，Jones の樹枝状アルゴリズムが Naranjo ら調査票と比べ質問項目が少なく簡略化されていることにも着目し，因果関係の評価に重要と考えられる質問項目を加えることで，より正確な評価を行えるようにした．

このようにして構築した改変樹枝状アルゴリズムを，薬学系学生と専門家を評価者として信頼性を評価した結果，学生，専門家共に κ 係数は良好な値を示した．一方，改変評価票においては，専門家では改変樹枝状アルゴリズムとほぼ同様の κ 係数を示したものの，学生による κ 係数は低かった．改変評価票で学生の信頼性評価が低かった理由としては，評価票が樹枝

状アルゴリズムと比べ簡便性において劣るためと考えられた．この改変評価票の信頼性を向上させるためには学生等，評価に不慣れな評価者に対しては事前に専門的トレーニングが必要であった可能性がある．すなわち，今回構築した改変樹枝状アルゴリズムは改変評価票と比較し，熟練性を要さずに精度よく使用できる簡便性があり，一般消費者から報告される有害事象報告を因果関係の確からしさに基づいて篩い分ける際の，ドラッグストア，医療機関，製造販売元，保健所等，職種の異なる臨床現場での有害事象評価におけるスクリーニングとしての活用が期待されると考えられた．

　今回の研究では，新たに構築した改変樹枝状アルゴリズムの信頼性評価を，すでに構築した改変評価票との比較により検討した．その理由は，高い多評価者間κ係数を報告した改変評価票[4]が，その後の追試験で異なる職種間に適用した場合，必ずしも満足のいく信頼性を示さなかったことによる[8]．そのため今回，異なる職種においても信頼性を保つ評価法を目指して改変樹枝状アルゴリズムの構築を行い，改変評価票との比較を行った．

　評価者により評価された健康食品の摂取に伴う有害事象の多くは，すでに改変評価票で報告した結果[4]と同様，因果関係の弱いカテゴリーである "possible" に集中した（Fig. 2）．この傾向が生じる理由としては，健康食品の情報のもつ不確かさ（曖昧性）が影響していると考えられる．そのような健康食品の有害事象の特殊性を踏まえ，今回の研究では，改変評価票のカテゴリー分類に準じて，Jones 改変アルゴリズムの "possible" を，"highly possible" と "possible" の2つに細分類した．さらに「時間的関連はあるが，他の要因による可能性が強いケース」を，他の要因が考えにくい他の "possible" のケースと区別するために "remote" に篩い分けた．その結果，「時間的関連はあるが，他の要因の可能性が強いケース」と「時間的関連がないケース」のいずれもが "remote" に含まれたことに関して議論の余地が生じた．また，健康食品の多くは均一な物質ではなく，さまざまな物質を含んでおり，その割合は生産ロットによって異なると予想されるが，本改変樹枝状アルゴリズムでは，製品間の不均一性に関する情報は含まれていない点，因果関係評価における限界を有している．これらの点に関しては，今後，改良の余地が残された．

　健康食品の摂取に伴う有害事象には，たとえば "摂取しない状態でも一定頻度で起こるような有害事象が

あり，そのような場合は頻度の増加を検出する必要性から，個別症例ではなく，同様の症例を集積して評価することが有用である．最近，自発報告等による大量の有害事象報告についてデータマイニング手法で安全性シグナルを検出し，その妥当性の検証や優先順位付けを行うことが，リスク管理の観点から重要視されつつある．そのような流れの中で，安全性シグナルの基となる情報の確からしさの評価として，本アルゴリズムの必要性は高いと考える．

結　論

　今回構築した改変樹枝状アルゴリズムは，改変評価票と同様の信頼性を有し，さらに臨床現場でのスクリーニングとして，健康食品の摂取に伴う健康被害の因果関係判定法として使用が可能であると考えられた．今後，実際に医療現場で使用する職種における評価での臨床的な有用性を検討する必要がある．

文　献

1) 梅垣敬三．健康食品の有害事象の事例と解説　有害事例の特徴．*Functional Food*. 2007；1(1)：90-5.

2) Agbabiaka TB, Savović J, Ernst E. Methods for causality assessment of adverse drug reactions：a systematic review. *Drug Saf*. 2008；31(1)：21-37.

3) 山田浩，松本圭司，清水雅之，熊谷翼，渡邉崇之，近都正幸ほか．健康食品摂取と健康上の有害事象の因果関係を評価するためのアルゴリズムの提案．臨床薬理．2009；40(4)：163-8.

4) 松本圭司，高橋光明，梅垣敬三，山田浩．健康食品摂取に伴う健康被害報告の因果関係評価法の構築：改定評価票による評価者間信頼性評価．臨床薬理．2011；42(4)：211-4.

5) 山田浩．健康食品の摂取に伴う健康被害として報告される情報の因果関係評価判定法の検討．第24回健康食品フォーラム報告書．医療経済研究・社会保険福祉協会．2011；48-62.

6) Naranjo CA, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, et al. A method for estimating the probability of adverse drug reactions. *Clin Pharmacol Ther*. 1981；30(2)：239-45.

7) Jones JK. Adverse drug reactions in the community health setting：approaches to recognizing, counseling, and reporting. *Fam Community Health*. 1982；5(2)：58-67.

8) 小野彩奈，高橋光明，一丸佳代，松本圭司，藤本和子，脇昌子ほか．健康食品摂取に伴う健康被害報告の因果関係評価法の構築：専門家による信頼性評価．第15回日本医薬品情報学会学術大会，大阪，2012年7月7-8日．

Hepatic cytochrome P450 mediates interaction between warfarin and *Coleus forskohlii* extract *in vivo* and *in vitro*

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Abstract

Objectives This study aimed to determine whether *Coleus forskohlii* extract (CFE) influences the anticoagulant action of warfarin in mice *in vivo* and its relationship with hepatic cytochrome P450 (CYP).

Methods Mice were fed various doses of CFE standardised with 10% forskolin in a normal diet for one week, or in protein diets containing 7% and 20% casein (low and normal) for four weeks. They were then administered with warfarin by gavage on the last two days of the treatment regimen, and blood coagulation parameters, as well as hepatic CYP, were analysed at 18 h after the last dose. Direct interaction between CFE and forskolin with CYP2C was evaluated *in vitro*.

Key findings CFE dose dependently increased hepatic total CYP content and *S*-warfarin 7-hydroxylase activity at a dietary level of ≥0.05%. Warfarin-induced anticoagulation was attenuated by CFE in parallel with CYP induction. The findings were similar in mice fed diets containing CFE and different ratios of protein. CFE directly inhibited CYP2C activity in mouse and human liver microsomes *in vitro*, whereas forskolin was only slightly inhibitory.

Conclusions CFE attenuates the anticoagulant action of warfarin by inducing hepatic CYP2C; thus, caution is required with the combination of warfarin and dietary supplements containing CFE.

Introduction

The use of herbal supplements has increased worldwide.[1] Such dietary supplements are perceived as safe because the ingredients are natural and they have been used for centuries in oriental cultures. However, adverse effects have been associated with herbal supplements as their use has increased. The causes of these adverse effects include contamination with pharmaceutical agents or toxic substances by poor manufacturing practices or by adulteration,[2,3] allergic reactions, and interactions with prescribed drugs.[4,5]

Among the causes of adverse effects, drug–herb interactions cause the most concern because consumers of herbal supplements often take prescribed drugs concomitantly[2,4,5] and health professionals might be unaware of possible interactions.[6,7] In addition, a decrease in efficacy or an increase in the adverse effects of prescribed drugs might interfere with appropriate medical care and have a fatal outcome. Interactions between some herbal ingredients, such as St John's wort[8] and ginkgo biloba,[9] have been documented, but those for other herbal ingredients remain unknown.

Weight-loss supplements are popular, but they can cause health problems.[10] *Coleus forskohlii* is a popular herbal ingredient for commercial weight-loss dietary supplements. *C. forskohlii* is native to India,[11] where it has been used for centuries in Ayurvedic medicine to treat various diseases of the cardiovascular, respiratory, gastrointestinal and central nervous systems.[12] Extracts of *C. forskohlii* (CFE) roots contain the diterpene forskolin, which increases cAMP concentrations via the activation of adenylate cyclase, resulting in various therapeutic effects against asthma and idiopathic congestive cardiomyopathy.[13,14] Theoretically, an increase in cAMP induced by forskolin will enhance lipolysis leading to elevated fat degradation and physiological fat utilisation, and thus promote fat and weight loss. In fact, forskolin increases both cAMP accumulation and lipolysis in fat cells,[15,16] and

CFE standardised with forskolin reduces fat accumulation in ovariectomised rats[17] and induces favorable effects on body fat in overweight women and obese men.[18,19]

We previously showed that feeding mice with a diet containing CFE (standardised with 10% forskolin) obviously dose- and time-dependently induced hepatic cytochrome P450 (CYP) enzymes.[20] Significant induction of the hepatic CYP content and CYP2C activity was evident at an intake dose of 0.05%; the CFE dose of 60 mg/kg body weight in mice corresponded to about 5 mg/kg body weight of a human equivalent dose when calculated using the body surface normalisation method.[21] Forskolin had little effect on CYP enzyme induction, indicating that an unknown factor is involved. These findings suggests that CFE interacts with prescribed drugs. However, whether CFE actually interacts with drugs *in vivo* remains unclear.

The oral anticoagulant, warfarin, interacts with various foods and drugs,[22,23] resulting in serious adverse events such as bleeding and thrombus. Warfarin generally comprises a racemic mixture of the two active enantiomers, *R*- and *S*-warfarin. The latter has powerful anticoagulant action[24,25] and is metabolised by the CYP2C subfamily of enzymes,[26] which CFE induces in mice.[20] Warfarin binds exclusively to albumin in the blood, and an increase in unbound warfarin due to a decrease in albumin enhances the anticoagulant action.[24] Plasma albumin is likely to decrease in individuals on a diet and weight-loss supplements containing CFE. We found that feeding rats with a low-protein diet induced hepatic CYP and decreased plasma albumin.[27] These changes in plasma albumin and CYP induction caused by the low-protein diet counteracted the influence of warfarin on anticoagulation.

This study evaluates the interaction of CFE with warfarin in mice *in vivo* in terms of hepatic CYP induction and the effect of a low-protein diet. We also examined the direct interaction between CFE and CYP2C enzymes in mouse and human liver microsomes *in vitro*. Our results clearly showed that CFE interacted with warfarin and attenuated the anticoagulant action of warfarin *in vivo*, and that CYP2C enzyme induction was involved in the mechanism of the interaction.

Materials and Methods

Materials

Powdered CFE standardised with 10% forskolin was prepared as follows. Dried roots of *C. forskohlii*, obtained from Bangalore in southern India, were crushed and supercritically extracted under CO_2 gas. The forskolin-rich extract (20–30%) was mixed with dextrin to a forskolin concentration of 10%. These processes were outsourced to Tokiwa Phytochemical Co. Ltd (Chiba, Japan). The CFE comprised: water, 5.6%; protein, 0.3%; lipids, 22.7%; ash, 2.2% and carbohydrates, 69.2%.

The components of the AIN93G semi-purified diet were purchased from Oriental Yeast Co. Ltd (Tokyo, Japan) and included cornstarch, vitamin-free casein, cellulose, mineral mixture (AIN93G) and vitamin mixture (AIN93G). The composition of the AIN93G semi-purified diet has been described by Reeves.[28] We analysed CYP2C enzymes using *S*-warfarin, 7-hydroxywarfarin, 7-ethoxycoumarin and diclofenac purchased from Sigma-Aldrich Inc. (St Louis, MO, USA), and NADPH from Oriental Yeast Co Ltd. The P450-Glo CYP2C9 Screening System (Luciferin-H) and NADPH regeneration systems were obtained from Promega Co. (Madison, WI, USA). Human liver microsomes pooled from 50 donors were obtained from Life Technologies Co. (Carlsbad, CA, USA). Reagents for blood coagulation tests were obtained from Sysmex Co. (Kobe, Japan). Forskolin and 1,9-dideoxyforskolin were obtained from Sigma-Aldrich Inc. and all other reagents were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan).

Animal experiments

In experiment 1, male ICR mice, four weeks old (CLEA Japan, Inc., Tokyo, Japan), were housed at a constant temperature $(23 \pm 1°C)$ under a 12-h light–dark cycle in polypropylene cages. After acclimatisation for one week, the mice were fed the AIN93G semi-purified diet and CFE (0, 0.01, 0.05 and 0.15%) for one week.

In experiment 2, a study on the effects of dietary protein, mice were fed for four weeks with a diet containing either 7% (low) or 20% (normal) casein protein based on the AIN93G semi-purified formula with CFE (0, 0.05 and 0.15%). Table 1 shows the composition of the two diets.

In both experiment 1 and 2, each group consisted of six mice. The mice were administered by intragastric gavage with warfarin racemate (1.0 and 0.25 mg/kg in experiments 1 and 2) dissolved in 0.5% carboxymethylcellulose or vehicle for the last two days of the treatment regimen. In experiment 2,

Table 1 Composition of experimental diets (g/kg)

Ingredient	Dietary protein level	
	Low-protein diet (7%)	Normal-protein diet (20%)
Vitamin-free casein	70	200
Corn starch	641.5	529.5
Cellulose	50	50
Sucrose	120	100
L-Cystine	1.05	3.00
Choline bitartate	2.5	2.5
Soybean oil (no additives)	70	70
Vitamin mixture (AIN93G)	10	10
Mineral mixture (AIN93G)	35	35
Tertiary butylhydroquinone	0.014	0.014

– 32 –

we selected a low dose of warfarin to detect slight changes that might be induced by diets containing different ratios of protein. The mice were anaesthetised with pentobarbital and killed at 18 h after the final administration of warfarin according to a report by Sato *et al.*[29] Blood was collected from the caudal vena cava into tubes containing 3.2% sodium citrate (1 : 9 dilution) for analysis of blood coagulation, and into other tubes for serum preparation. The livers were immediately removed, weighed, snap frozen with dry ice and stored at −80°C.

All procedures were in accordance with the National Institute of Health and Nutrition guidelines for the Care and Use of Laboratory Animals, and approved by the ethical committee in the National Institute of Health and Nutrition (No. 1011, May 10th, 2010).

Analytical methods

High-performance liquid chromatography analysis of *Coleus forskohlii* extract

The CFE used in this study was characterised by HPLC equipped with UV detection (at 210 nm) and evaporative light scattering detection (ELSD). The HPLC conditions and sample preparation were basically according to a validated HPLC method described elsewhere.[30] Briefly, CFE sample extracted with acetonitrile was injected into HPLC-UV-ELSD. HPLC apparatus was a Shimadzu HPLC-VP system (Shimadzu Corporation, Kyoto, Japan). The sample was applied to an L-column ODS, 4.6×250 mm, 5μm particle size (Chemical Inspection & Testing Institute, Tokyo, Japan) at 35°C and eluted with a linear gradient of water (A) and acetonitrile (B). The gradient protocol was 0–12.0 min, 50–80% B; 12.1–39.0 min, 100% B at a flow rate of 1.2 ml/min. Forskolin and 1,9-dideoxyforskolin in the sample were quantified by HPLC-ELSD. The actual content of forskolin and 1,9-dideoxyforskolin was 10.37 g/100 g and 1.21 g/100 g, respectively.

Analysis of cytochrome P450 content and activity

The liver was rinsed with 0.9% (w/v) NaCl, homogenised in 50 mmol/l Tris-HCl buffer (pH 7.4) containing 0.25 mol/l sucrose and separated by centrifugation at 10 000*g* at 4°C for 30 min. The supernatant was clarified by centrifugation at 105 000*g* at 4°C for 60 min and used as microsomes to determine the CYP levels. The total CYP content and the activity of the CYP2C subtype enzyme as *S*-warfarin 7-hydroxylase were determined as described.[31] We investigated CYP2C-specific inhibition (experiment 3) using the 6'deoxyluciferin (Luciferin-H) provided in the P450-Glo assay (Promega), with untreated microsomes from mouse and human livers as enzyme sources and diclofenac as a positive inhibitor, according to the manufacturer's instructions. Luminescent signals from the reaction were measured by luminometry

(GloMax96 Microplate Luminometer; Promega), and the inhibitory activity of CFE, forskolin or diclofenac on CYP2C enzyme was determined as ratio (%) of treatment with vehicle. CYP2C activity was measured using various concentrations of CFE and Luciferin-H substrate to construct Dixon plots. Activity was expressed as relative light units (RLU)/mg protein/min.

Other analyses

Plasma samples were immediately centrifuged at 4320*g* at 4°C for 10 min. Coagulation parameters (prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombotest Owren (TTO)) were measured using an automated blood coagulation analyser (CA-50; Sysmex) according to the manufacturer's protocol. PT and TTO are indicators of the extrinsic and common pathways of the coagulation cascade, respectively, and are used to monitor warfarin therapy. On the other hand, APTT is an indicator of both the intrinsic and common pathways of the coagulation cascade. Protein concentrations were determined using BCA protein assay kits (Pierce, Rockford, IL, USA). Serum albumin was determined using the A/G *B*-test Wako (Wako Pure Chemical Industries).

Statistical analyses

Data are presented as means and standard error (SE) for individual groups and were statistically analysed using one-way (experiment 1) and two-way (experiment 2) analysis of variance with Tukey's multiple comparison test or Student's *t*-test when two groups were compared. Differences at $P < 0.05$ were considered significant. All statistical analyses were performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA).

Results

High-performance liquid chromatography profile of *Coleus forskohlii* extract

CFE is a natural plant product and the components may vary due to the extraction and preparation methods. Thus, to characterise the profile of CFE sample used in this study, the sample was injected into HPLC-UV-ELSD. Many peaks were observed in the chromatogram of HPLC-UV detection, while four peaks were observed in the chromatogram of HPLC-ELSD (Figure 1). The content of forskolin and 1,9-dideoxyforskolin in the CFE sample was 10.37% and 1.21%, respectively.

Interaction between *Coleus forskohlii* extract and warfarin in blood coagulation (experiment 1)

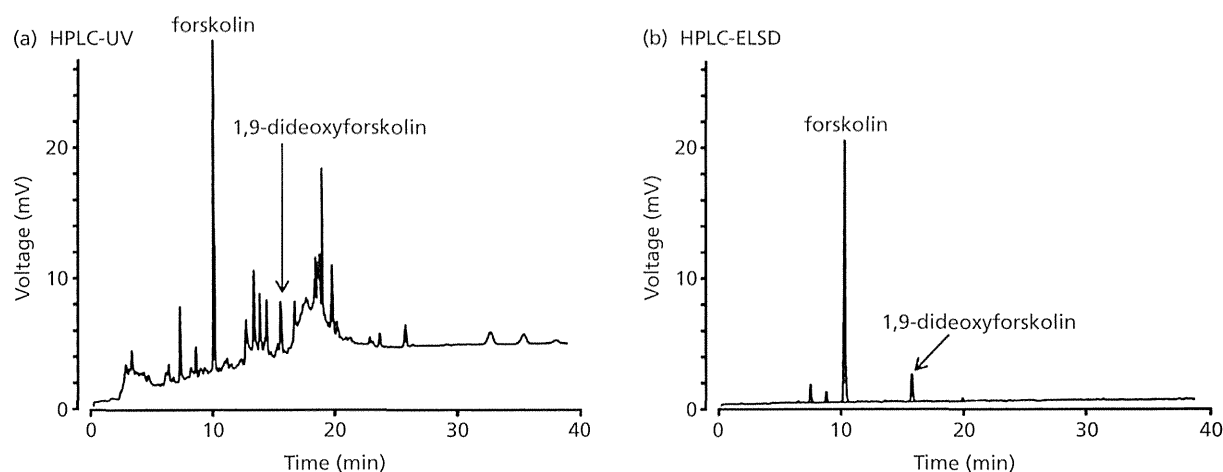One week of dietary CFE dose dependently increased the total hepatic CYP content and activity of *S*-warfarin

Figure 1 Typical HPLC chromatograms of the *Coleus forskohlii* extract (CFE) used in this study. CFE sample extracted with acetonitrile was injected into HPLC with UV detection (at 210 nm) and evaporative light scattering detection (ELSD). Details of conditions are given in Methods.
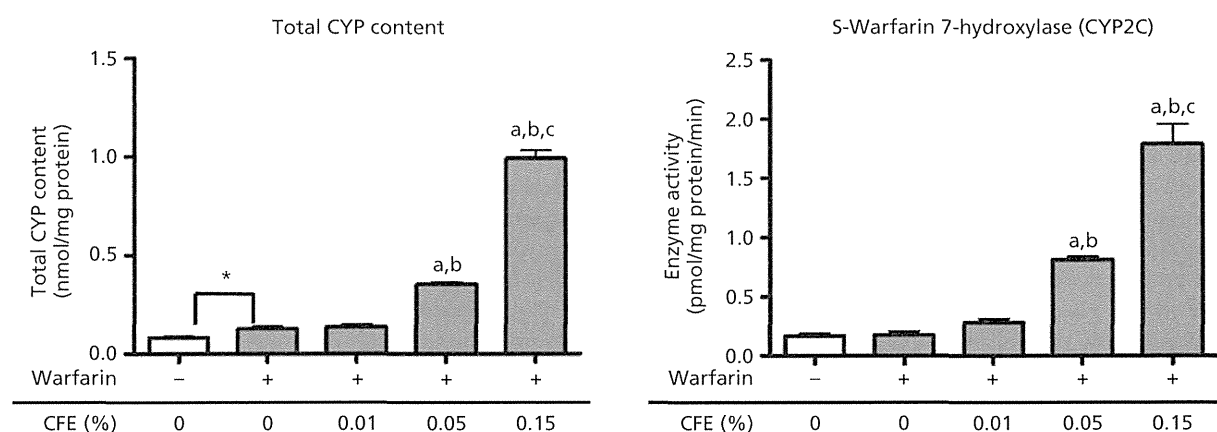

Figure 2 Changes in total cytochrome P450 content and *S*-warfarin 7-hydroxylase in livers of mice administered with various doses of *Coleus forskohlii* extract (CFE) and warfarin (experiment 1). Values are expressed as means and SE, $n = 6$. *$P < 0.05$, compared with CFE (0%) without warfarin. $^{a}P < 0.05$, compared with CFE (0%) with warfarin. $^{b}P < 0.05$, compared with CFE (0.01%) with warfarin. $^{c}P < 0.05$, compared with CFE (0.05%) with warfarin.

7-hydroxylase, a CYP2C enzyme. Significant induction was evident at dietary CFE doses above 0.05%, which corresponded to a dose of 72 mg/kg body weight (Figure 2). Liver weight significantly increased at a CFE dose of 0.15% (Table 2). Warfarin alone (1 mg/kg) for the last two days of the treatment regimen slightly increased the total CYP content, but did not induce *S*-warfarin 7-hydroxylase activity. The anticoagulant effect of warfarin, evaluated by blood coagulation parameters (PT, APTT and TTO), was dose-dependently attenuated by CFE (Figure 3), which corresponded with the induction of CYP enzymes.

Effect of dietary protein on interaction between warfarin and *Coleus forskohlii* extract (experiment 2)

Various doses of CFE in the 7% or 20% casein diets for four weeks dose-dependently induced hepatic total CYP content and the activity of *S*-warfarin 7-hydroxylase in mice. The overall induction was higher than that in experiment 1. This would be due to the longer period of CFE administration (Figure 4). We selected a low dose of warfarin to detect slight changes that might be induced by diets containing different ratios of protein. Warfarin (0.25 mg/kg) administration for

Table 2 Changes in body weight, liver weight and food intake of mice administered various doses of *Coleus forskohlii* extract (CFE) and warfarin (experiment 1)

CFE treatment	0%	0%	0.01%	0.05%	0.15%
Warfarin treatment	–	+	+	+	+
Final body weight (g)	34.4 ± 0.9	34.1 ± 0.7	33.7 ± 0.6	34.2 ± 0.7	35.0 ± 0.5
Liver weight (%/body weight)	5.84 ± 0.10	5.72 ± 0.18	5.23 ± 0.18	5.88 ± 0.18	6.87 ± 0.17[a,b,c]
Average dairy food intake (g)	4.9 ± 0.07	4.9 ± 0.06	4.9 ± 0.05	4.9 ± 0.10	4.9 ± 0.09

Values are expressed as means ± SE, $n = 6$. *$P < 0.05$, compared with CFE (0%) without warfarin. [a]$P < 0.05$, compared with CFE (0%) with warfarin. [b]$P < 0.05$, compared with CFE (0.01%) with warfarin. [c]$P < 0.05$, compared with CFE (0.05%) with warfarin.
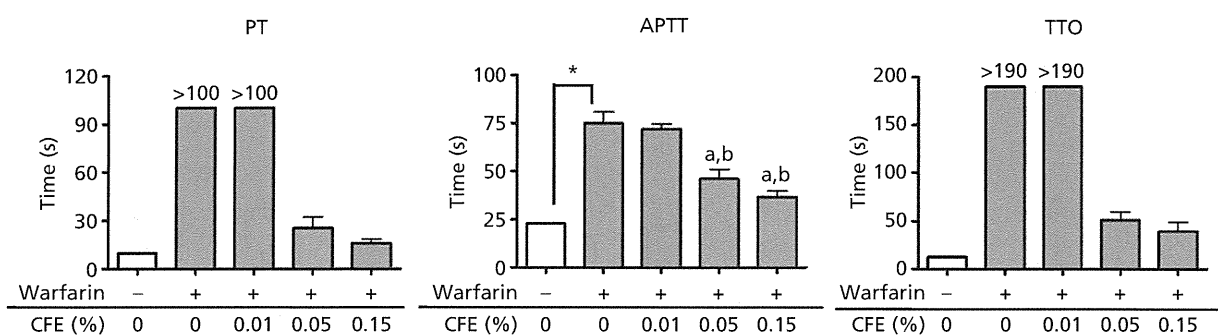
Figure 3 Changes in warfarin-induced blood coagulation parameters in mice administered with various doses of *Coleus forskohlii* extract (CFE) and warfarin (experiment 1). Coagulation parameters are prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombotest Owren (TTO). Values are expressed as means and SE, $n = 6$. *$P < 0.05$, compared with CFE (0%) without warfarin. [a]$P < 0.05$, compared with CFE (0%) with warfarin. [b]$P < 0.05$, compared with CFE (0.01%) with warfarin.
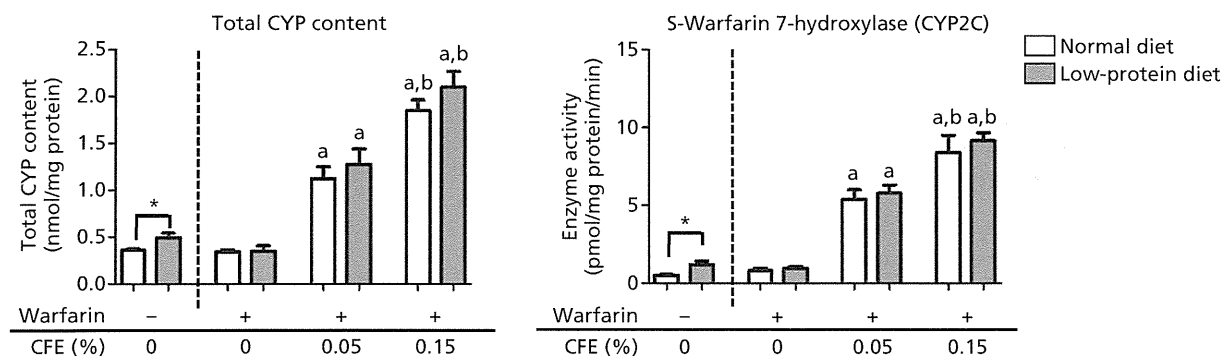
Figure 4 Changes in total cytochrome P450 content and *S*-warfarin 7-hydroxylase in livers of mice administered with various doses of *Coleus forskohlii* extract (CFE) in low (7%) or normal (20%) protein diets and warfarin (experiment 2). Values are expressed as means and SE, $n = 6$. *$P < 0.05$, compared with CFE (0%) without warfarin. [a]$P < 0.05$, compared with CFE (0%) with warfarin. [b]$P < 0.05$, compared with CFE (0.01%) with warfarin.

the last two days of the regimen did not affect such changes. The amount of dietary protein affected the groups that were not treated with warfarin; the effect was obvious in the group administered with the low, compared with the normal, protein diet. However, the effect was less clear in the groups treated with warfarin and CFE. Body weight was lower in the group fed the low, rather than the normal, protein diet

(Table 3), but the plasma albumin concentration did not differ between the two groups at any time. The magnitude of warfarin-induced blood coagulation parameters decreased in the groups administered with CFE, but the effect of dietary protein was unclear (Figure 5). The decrease in blood anticoagulation parameters corresponded to the increase in CYP enzyme induction.

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Table 3 Changes in body weight, liver weight, serum albumin concentration and food intake of mice administered with various doses of *Coleus forskohlii* extract (CFE) in low (7%) or normal (20%) protein diet and warfarin (experiment 2)

	Normal protein diet (20% casein)				Low-protein diet (7% casein)			
Warfarin treatment	–	+	+	+	–	+	+	+
CFE	0%	0%	0.05%	0.15%	0%	0%	0.05%	0.15%
Final body weight (g)	41.4 ± 1.5	39.4 ± 1.7	41.4 ± 1.3	41.1 ± 1.2	37.1 ± 1.2	35.7 ± 1.2	37.4 ± 1.3	37.9 ± 1.4
Liver weight (%/body weight)	3.86 ± 0.14	3.91 ± 0.11	5.71 ± 0.21[a]	8.01 ± 0.48[a,b]	3.73 ± 0.22	4.38 ± 0.31	4.99 ± 0.05	5.95 ± 0.14[a,b]
Serum albumin (Concentration, g/dl)	3.26 ± 0.16	3.12 ± 0.21	3.13 ± 0.19	3.06 ± 0.24	3.48 ± 0.15	3.62 ± 0.12	3.11 ± 0.07	2.96 ± 0.18
Average dairy Food intake (g)	4.8 ± 0.13	4.7 ± 0.11	4.6 ± 0.06	4.6 ± 0.10	4.8 ± 0.04	4.7 ± 0.06	4.8 ± 0.06	4.6 ± 0.09

Values are expressed as means ± SE, $n = 6$. [a]$P < 0.05$, compared with (0%) with warfarin in the same protein diet. [b]$P < 0.05$, compared with CFE (0.05%) with warfarin in the same protein diet.
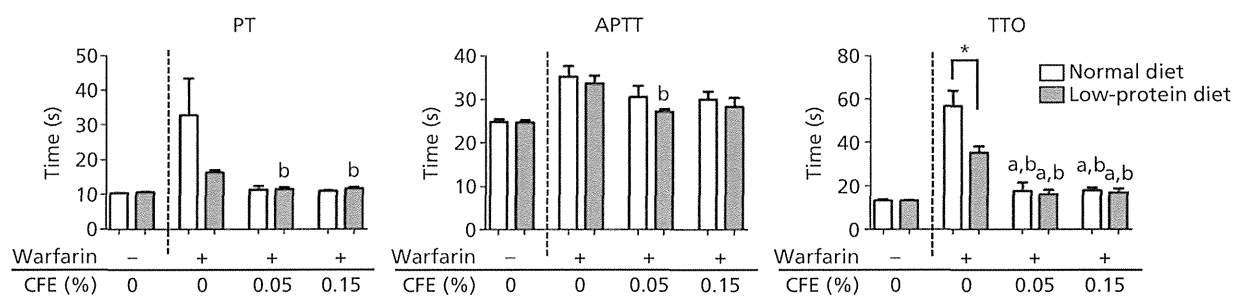
Figure 5 Changes in warfarin-induced blood coagulation parameters in mice administered with various doses of *Coleus forskohlii* extract (CFE) in low (7%) or normal (20%) protein diets and warfarin (experiment 2). Coagulation parameters are as described in the legend to Figure 2. Values are expressed as means and SE, $n = 6$. *$P < 0.05$, compared with CFE (0%) without warfarin. [a]$P < 0.05$, compared with CFE (0%) with warfarin. [b]$P < 0.05$, compared with CFE (0.01%) with warfarin.

Direct interaction of *Coleus forskohlii* extract on CYP2C enzyme in human and mouse microsomes *in vitro* (experiment 3)

We compared the direct interaction of CFE with CYP2C enzymes between mouse and human liver microsomes. We found that CFE dose-dependently inhibited CYP2C activity in all microsomes, with a 50% inhibitory concentration (IC_{50}) value of 7 and 9 µg/ml for mice and humans, respectively (Figure 6). Under these conditions, the IC_{50} of diclofenac, which is a positive control that commonly serves as a probe for CYP2C9 assays in humans, was similar between the mouse and human microsomes, at 8 and 10 µg/ml, respectively. The inhibitory effect of CFE on mouse CYP2C was characterised in a Dixon plot (Figure 7), which showed an approximate Ki value of 7.5 µg/ml. To clarify the contribution of forskolin on the inhibitory effect, 25 µg/ml of CFE and 2.5 µg/ml of forskolin, which was equivalent to the amount in the CFE, were added to the CYP2C assay. Pure forskolin was slightly inhibitory, whereas CFE was more so in both mouse and human liver microsomes. The values (% of control activity, means ± SE of four determinations) for forskolin vs CFE
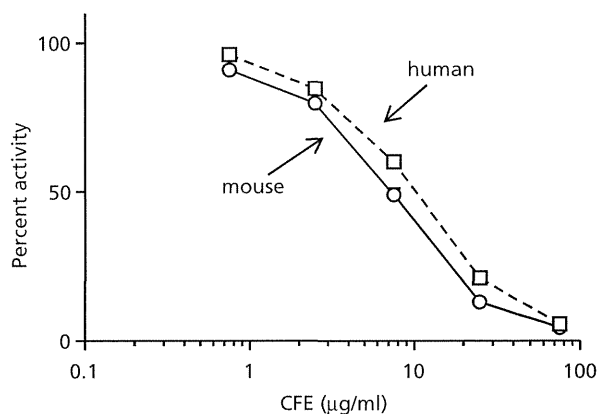
Figure 6 Inhibitory effect of *Coleus forskohlii* extract (CFE) on CYP2C activity of mouse and human liver microsomes (experiment 3). CYP2C activity was determined in presence of 0.75–75 µg/ml CFE. Values are expressed as means and SE of four determinations.
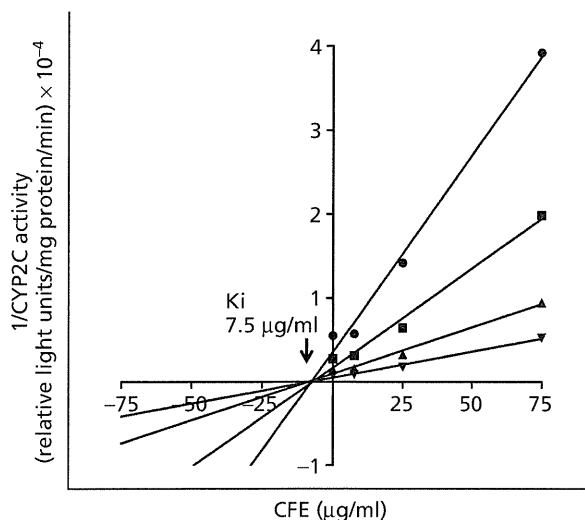
Figure 7 Effect of *Coleus forskohlii* extract (CFE) on CYP2C activity in mouse liver microsomes *in vitro* (experiment 3). Dixon plot shows CYP2C activity measured in presence of 7.5–75 μg/mL of CFE in mouse liver microsomes *in vitro*. Loaded 6'deoxyluciferin (Luciferin-H) concentrations: ●, 50 μM; ▦, 100 μM; ▲, 200 μM; ▼, 400 μM. Points represent means of three determinations.

in mouse and human microsomes were 75 ± 1.3 vs 12 ± 1.0 and 92 ± 3.3 vs 17 ± 0.3 (both $P < 0.01$), respectively.

Discussion

CFE contains forskolin, which activates adenylate cyclase and thus induces various pharmacological effects. Currently, CFE standardised with 10% forskolin is a popular herbal ingredient of dietary weight-loss supplements. Here, we showed that CFE attenuated the anticoagulant action of warfarin *in vivo*, and that hepatic total CYP and *S*-warfarin 7-hydroxylase induced by CFE, but not by forskolin, were involved in the mechanism of action. Experiment 1 showed that warfarin interacted with CFE at doses of approximately 70 mg/kg body weight for one week. This dose in mice corresponds to about 6 mg/kg in humans calculated using the body surface normalisation method.[21] Notably, the calculated human dose was within the range of CFE intake from commercial dietary weight-loss supplements, suggesting that the intake of warfarin together with dietary supplements containing CFE increases the risk of thrombus formation.

Consumers of dietary weight-loss supplements containing CFE might also be on extreme diets, which could result in their having a low-protein nutritional status. Warfarin binds exclusively to albumin in the blood, and an increase in unbound warfarin resulting from a decrease in albumin might enhance the anticoagulant action of CFE.[24] On the other hand, we found that feeding a low-protein diet to rats

increases hepatic CYP levels,[27] which might attenuate the anticoagulant action of warfarin. Thus, precisely how CFE interacts with warfarin under conditions of a low-protein diet should be determined. Our results showed that various doses of CFE administered over a period of four weeks dose-dependently diminished the anticoagulant action of warfarin in mice fed diets containing low and normal amounts of protein. The diminished anticoagulant action of warfarin corresponded to the induction of total CYP and *S*-warfarin7-hydroxylase activity, which were slightly higher in the mice fed the low-protein diet. The effect of this diet on the interaction between CFE and warfarin and the effect on anticoagulation was less clear, perhaps because of the following. The induction of CYP by four weeks of CFE administration was so obvious that it obscured the effect of the low-protein diet. Furthermore, plasma albumin was notably unaffected by the low-protein diet under our experimental conditions.

The CYP2C subfamily is associated with *S*-warfarin metabolism in humans and mice.[32] However, whether or not the pathways of CFE interaction with warfarin are the same in humans and mice *in vivo* remained unclear. We therefore evaluated the direct interaction between CFE and forskolin on CYP2C enzyme using the CYP2C9 Screening System in mouse and human liver microsomes *in vitro*. The results showed that CFE inhibits CYP2C activity in human and mouse microsomes to a similar extent, whereas forskolin only slightly inhibited the activity. The inhibitory potency of forskolin was considerably lower than that of CFE containing 10% forskolin. This finding was consistent with those of our previous in-vivo mouse study,[20] which showed that an unidentified substance in CFE induces CYP enzymes *in vivo* and inhibits them *in vitro*. These findings imply that the unidentified substance is a substrate of the CYP2C enzyme. The IC$_{50}$ of diclofenac, a substrate for human CYP2C9, and of CFE were comparable between human and mouse liver microsomes in this study. This observation indicates that CFE interacts with other drugs, such as warfarin, that are metabolised by CYP2C9 in humans. We characterised CFE interaction with CYP2C in mouse liver microsomes using Dixon plots. The Ki value was 7.5 μg/ml and the inhibition was non-competitive. However, to conclude the type of inhibition would be difficult at present because CFE has many components, including forskolin, which might be involved in the inhibitory reaction in liver microsomes. If the substance associated with CYP induction *in vivo* and inhibition *in vitro* could be identified and isolated, the Ki value would be lower and the CFE mode of action would be clearer.

Although CFE is generally standardised with 10% forskolin as an active substance, the contribution of forskolin to the interaction of CFE with warfarin was negligible in this study and in our previous study.[20] Accordingly, the substance causing CYP induction must be identified and eliminated from CFE preparations for their safe inclusion in dietary

weight-loss supplements. There are many substances in the CFE, and it is unknown whether the inducer of CYP2C enzyme is a single component at present. Ding and Staudinger reported that forskolin and 1,9-dideoxyforskoiln, a nonadenylate cylcase-activating analogue, induced *CYP3A* gene expression through Pregnane X receptor (PXR) in cultured hepatocytes.[33] The CFE materials used in the present study contained 1.21% of 1,9-dideoxyforskolin. Therefore, 1,9-dideoxyforskolin may be a candidate involved in *CYP2C* induction. However, the intestinal absorption of 1,9-dideoxyforskoiln as well as forskolin is unknown. The use of weight-loss supplements seem to be higher in women than in men, and it is important to clarify whether sex difference exists in the induction of CYP by CFE intake. The assay system used in this study would be helpful to identify the active substances and in future detailed study.

Theoretically, CFE is thought to induce bleeding when taken with antiplatelet drugs, because the forskolin in CFE inhibits platelet functions.[34,35] The findings of our previous study[20] seemed to contradict this theory, since CFE induced both CYP3A and CYP2C, which catalyses 50% and 20% of prescribed drugs, respectively.[36,37] The induction of CYP by CFE attenuated the effect of warfarin in the present study, and would also diminish the effects of antiplatelet drugs, thereby resulting in an increased risk of thrombus formation. Healthcare professionals should not blindly accept existing information, but should observe and communicate with patients who are receiving warfarin, antiplatelet drugs or other drugs metabolised by CYP2C and CYP3A while consuming dietary weight-loss supplements containing CFE.

Conclusions

Coleus forskohlii extract induced CYP2C and diminished the anticoagulant property of warfarin in mice *in vivo*. We also showed that CFE inhibited CYP2C from mouse and human liver microsomes *in vitro*, whereas forskolin did not. The substance involved in CYP induction *in vivo* and inhibition *in vitro* remains undefined.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

References

1. Gershwin ME *et al*. Public safety and dietary supplementation. *Ann N Y Acad Sci* 2010; 1190: 104–117.

2. Bent S, Ko R. Commonly used herbal medicines in the United States: a review. *Am J Med* 2004; 116: 478–485.

3. van Breemen RB *et al*. Ensuring the safety of botanical dietary supplements. *Am J Clin Nutr* 2008; 87: 509S–513S.

4. Izzo AA, Ernst E. Interactions between herbal medicines and prescribed drugs: an updated systematic review. *Drugs* 2009; 69: 1777–1798.

5. Ulbricht C *et al*. Clinical evidence of herb–drug interactions: a systematic review by the natural standard research collaboration. *Curr Drug Metab* 2008; 9: 1063–1120.

6. Sleath B *et al*. Ethnicity and physician-older patient communication about alternative therapies. *J Altern Complement Med* 2001; 7: 329–335.

7. Giveon SM *et al*. Are people who use 'natural drugs' aware of their potentially harmful side effects and reporting to family physician? *Patient Educ Couns* 2004; 53: 5–11.

8. Zhou SF, Lai X. An update on clinical drug interactions with the herbal antidepressant St. John's wort. *Curr Drug Metab* 2008; 9: 394–409.

9. Uchida S *et al*. Effects of Ginkgo biloba extract on pharmacokinetics and pharmacodynamics of tolbutamide and midazolam in healthy volunteers. *J Clin Pharmacol* 2006; 46: 1290–1298.

10. Pittler MH *et al*. Adverse events of herbal food supplements for body weight reduction: systematic review. *Obes Rev* 2005; 6: 93–111.

11. Bhat SV *et al*. Structures and stereochemistry of new labdane diterpenoids from Coleus forskohlii briq. *Tetrahedron Lett* 1977; 19: 1669–1672.

12. Ammon HP, Muller AB. Forskolin: from an ayurvedic remedy to a modern agent. *Planta Med* 1985; 51: 473–477.

13. Bauer K *et al*. Pharmacodynamic effects of inhaled dry powder formulations of fenoterol and colforsin in asthma. *Clin Pharmacol Ther* 1993; 53: 76–83.

14. Baumann G *et al*. Cardiovascular effects of forskolin (HL 362) in patients with idiopathic congestive cardiomyopathy–a comparative study with dobutamine and sodium nitroprusside. *J Cardiovasc Pharmacol* 1990; 16: 93–100.

15. Allen DO *et al*. Relationships between cyclic AMP levels and lipolysis in fat cells after isoproterenol and forskolin stimulation. *J Pharmacol Exp Ther* 1986; 238: 659–664.

16. Okuda H *et al*. Relationship between cyclic AMP production and lipolysis induced by forskolin in rat fat cells. *J Lipid Res* 1992; 33: 225–231.

17. Han LK *et al*. Effects of Coleus forskohlii on fat storage in ovariectomized rats. *Yakugaku Zasshi* 2005; 125: 449–453.

18. Henderson S et al. Effects of coleus forskohlii supplementation on body composition and hematological profiles in mildly overweight women. J Int Soc Sports Nutr 2005; 2: 54–62.

19. Godard MP et al. Body composition and hormonal adaptations associated with forskolin consumption in overweight and obese men. Obes Res 2005; 13: 1335–1343.

20. Virgona N et al. Coleus forskohlii extract induces hepatic cytochrome P450 enzymes in mice. Food Chem Toxicol 2011; 50: 750–755.

21. Reagan-Shaw S et al. Dose translation from animal to human studies revisited. FASEB J 2008; 22: 659–661.

22. Holbrook AM et al. Systematic overview of warfarin and its drug and food interactions. Arch Intern Med 2005; 165: 1095–1106.

23. Fugh-Berman A, Ernst E. Herb-drug interactions: review and assessment of report reliability. Br J Clin Pharmacol 2001; 52: 587–595.

24. Palareti G, Legnani C. Warfarin withdrawal. Pharmacokinetic-pharmacodynamic considerations. Clin Pharmacokinet 1996; 30: 300–313.

25. Taki Y et al. Ginkgo biloba extract attenuates warfarin-mediated anticoagulation through induction of hepatic cytochrome P450 enzymes by bilobalide in mice. Phytomedicine 2011; 19: 177–182.

26. Kumar V et al. CYP2C9 inhibition: impact of probe selection and pharmacogenetics on in vitro inhibition profiles. Drug Metab Dispos 2006; 34: 1966–1975.

27. Taki Y et al. Effects of Ginkgo biloba extract on the pharmacokinetics and pharmacodynamics of tolbutamide in protein-restricted rats. J Pharm Pharmacol 2011; 63: 1238–1243.

28. Reeves PG et al. 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. J Nutr 1993; 123: 1939–1951.

29. Sato K et al. Comparison of the anticoagulant and antithrombotic effects of YM-75466, a novel orally-active factor Xa inhibitor, and warfarin in mice. Jpn J Pharmacol 1998; 78: 191–197.

30. Virgona N et al. A rapid HPLC with evaporative light scattering method for quantification of forskolin in multiherbal weight-loss solid oral dosage forms. Pharmazie 2010; 65: 322–326.

31. Umegaki K et al. Ginkgo biloba extract markedly induces pentoxyresorufin O-dealkylase activity in rats. Jpn J Pharmacol 2002; 90: 345–351.

32. Martignoni M, ed. Species and Strain Differences in Drug Metabolism in Liver and Intestine, University of Groningen, 2006.

33. Ding X, Staudinger JL. Induction of drug metabolism by forskolin: the role of the pregnane X receptor and the protein kinase a signal transduction pathway. J Pharmacol Exp Ther 2005; 312: 849–856.

34. Christenson JT et al. The effect of forskolin on blood flow, platelet metabolism, aggregation and ATP release. Vasa 1995; 24: 56–61.

35. Agarwal KC et al. Significance of plasma adenosine in the antiplatelet activity of forskolin: potentiation by dipyridamole and dilazep. Thromb Haemost 1989; 61: 106–110.

36. Tompkins LM, Wallace AD. Mechanisms of cytochrome P450 induction. J Biochem Mol Toxicol 2007; 21: 176–181.

37. Rendic S, Di Carlo FJ. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. Drug Metab Rev 1997; 29: 413–580.

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Coleus forskohlii エキス中の肝シトクローム P450 誘導物質の推定

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Estimation of Components which Induce Mice Cytochrome P450 in *Coleus forskohlii* Extract

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Drug-herb interaction is a major concern for the safety use of herbal products. In our previous mice study, we found that *Coleus forskohlii* extract (CFE) markedly induced hepatic cytochrome P450 (CYP), especially CYP2B, 2C and 3A type at the dose added to weight loss supplements. Also, we showed that forskolin, an active constituent in CFE, was not involved in the CYP induction *in vivo*. The present study was designed to estimate the compounds inducing CYP. CFE was fractionated into 4 (diethyl ether-, ethyl acetate-, acetone-, the remainder), and the effect of those fractions on CYP was examined in two systems: test materials were fed to mice for 2 weeks (*in vivo* system), and those were directly added to CYP3A enzyme assay (*in vitro* system). It was found that CYP inducing activity *in vivo* was mainly distributed in the diethyl ether-fraction, which also showed a direct inhibition of CYP3A activity *in vitro*. The water soluble fraction showed neither CYP induction *in vivo* nor CYP3A inhibition *in vitro*. It was also suggested the existence of several compounds inducing CYP, some of which was eliminated during the fractionation procedure. CFE-induced CYP induction *in vivo* was well correlated with an increase in liver weight, and was related to direct inhibition of CYP enzyme activity *in vitro*. Combination of these characteristics would be useful for further study to identify the active constituents in CFE materials that induce CYP.

Keywords: *Coleus forskohlii* / hepatic CYP / forskolin / herb / weight loss supplement

緒　言

インド地方の熱帯や亜熱帯地域に自生するシソ科の植物 *Coleus forskohlii* は，伝統医学アーユルベーダにおいて，心血管疾患や中枢神経系疾患，呼吸器疾患，腎疾患などの治療に用いられてきた(de Souza，et al., 1983). *Coleus forskohlii* の薬理作用は，根に含まれるジテルペンの forskolin がアデ

ニル酸シクラーゼを活性化して cAMP 濃度を上昇させることにより発現すると考えられている(Bauer，et al., 1993; Baumann，et al., 1990). Forskolin による cAMP 濃度の上昇は，ホルモン感受性リパーゼを活性化して脂肪分解を促進することから(Allen，et al., 1986)，*Coleus forskohlii* エキス (CFE) は，ダイエット効果が期待できるサプリメント素材として多用されている (Godard，et al., 2005). 近年のダイエットブームと，ハーブ類は「天然だから安全」との消費者のイメージから，CFE を含むサプリメントの利用者は益々

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