厚生科学研究費補助金（食品の安全確保推進研究事業）
（分担）研究報告書

健康食品の摂取に伴う有害事象の収集法に関する検討

研究分担者　山田　浩　　静岡県立大学薬学部
研究協力者　川﨑洋平　　静岡県立大学薬学部
　　井出和希　　静岡県立大学薬学研究院
　　野口真里茄　静岡県立大学薬学部

研究要旨

健康食品の摂取に関連した被害が発生した場合、保健所や消費者センターに情報が集約される。保健所に集まる情報は、主に医療機関を介して提供される情報であり、医学的検証がなされた事例が多い一方で、情報数が極めて限られていることが問題となっている。

本分担研究は、健康食品の利用者から健康被害の相談を受ける立場である診療所の医師、保険薬局の薬剤師にアンケート調査を行なうことにより、診療所、保険薬局から保健所に報告する際の支障の有無とその原因について調査することを目的に実施した。

医師・薬剤師各850名に郵送で無記名式アンケートを配布、588名（34.6%）から回答を得た。結果、保健所へ報告した経験を持つ医師、薬剤師は非常に少数であることが明らかとなった。報告しなかった理由として、健康食品が原因と断定できなかった、という理由を挙げる医師、薬剤師が最も多かった。報告にあたり支障になる事として、因果関係の判別が難しいという意見が71.5%の医師、82.5%の薬剤師から得られた。併せて、報告フォーマットの必要性について意見が寄せられた。

聴取の工夫や因果関係評価手法の利活用と共に、報告フォーマットの作成や報告手段について情報伝達を行うことで、保健所への報告状況の改善が見込まれる。

A. 研究目的

食品の中で健康効果を期待させる種々の製品は、一般に健康食品あるいはサプリメントと呼ばれており、利用者は年々増加している。それに伴い、健康食品の摂取に関連した健康被害の報告も増加しており、迅速な情報の収集と対応が不可欠である。

健康食品に関係した被害報告の主な情報としては、保健所を介して厚生労働省に集約される情報（保健所情報）、消費者センターから国民生活センターに集約される全国消費生活情報ネットワーク・システム情報（PIONET情報）、各企業が独自に収集している情報（企業情報）がある。PIONET情報および企業情報は主に消費者本人から直接提供された情報であり、報告件数は多い。しかし、医療機関を介しての情報ではなく、医学的信頼性に欠ける。一方、保健所情報は、主に医療機関を介して提供される情報であり、医学的検証がなされた事例が多いものの、現在、情報数が極めて限られている。すなわち、健康食品の摂取に伴う健康被害報告は保健所を介して厚生労働省に集約されるシステムがあるにも関わらず、その利活用がほとんど行われていないのが現状である。

本研究は診療所、保険薬局から保健所に情報を報告するにあたっての支障の有無、その原因の調査および具体的な改善策を検討することを目的として実施した。

B. 研究方法

健康食品利用者からの健康被害情報の相談を受ける立場である診療所の医師、保険薬局の薬剤師を対象に無記名のアンケート調査を行なった。

〈調査時期〉

平成27年11月3日（アンケート送付日）から平成28年1月末日まで

〈配布・回収方法〉

ランダム抽出にて調査対象となった施設にアンケート票を郵送し、任意で各施設に回答を求めた。調査結果の公表に関する同意取得はアンケート調査に対する回答と共に行ない、回答は無記名とした。診療所には1施設あたり1枚、薬局には1施設あたり2枚（2名以上の薬剤師が勤務することを想定）のアンケートを送付した。なお本研究は調査開始前に、静岡県立大学倫理審査委員会の承認を

得た。
〈調査対象者数の設定根拠〉
当研究室で実施した「平成 20 年度診療報酬の改定等に伴う後発医薬品の使用状況に関する保険薬局へのアンケート調査（*臨床薬理* 40:295-302, 2009)」の回収率に基づき対象者数を設定した。当該アンケート調査の回収率は 45.3%であった。本研究においても同地域の医療従事者（薬剤師等）を対象とすることから同様の回収率を見込んだ。配布するアンケートの、ある項目に対する有効回答割合の誤差を 5%以内に収めるためには 385 件の有効回答を得る必要がある。以上の点を考慮し、医師・薬剤師各 850 名にアンケートを配布することとした。
〈解析方法〉
選択形式の項目に関しては件数および割合を算出した。自由記載の項目に関しては、項目ごとに記載内容をまとめた。集計には、統計解析ソフト SAS version 9.4 for Windows (SAS Institute Inc. Cary, NC)を用いた。

C. 研究結果

〈回収結果〉
送付数に対する回収率と送付施設数に対する回収率をそれぞれ算出した。送付数に対する回収率は全体で 34.7%、送付施設数に対する回収率は全体で 39.6%であった。
〈背景情報〉
医師は全体の 89.1%が男性、薬剤師は男性、女性がほぼ同数となった。職務経験は、20 年以上の診察経験をもつ医師が全体の 90.4%、また 10 年以上の実務経験をもつ薬剤師が 37.4%を占め、実務経験の長い医師、薬剤師がアンケートに回答している傾向が見られた。 医師の診療科は内科が最も多く、内科、小児科、産科婦人科以外の診療科では整形外科、外科、耳鼻咽喉科が多いという結果となった。
〈回答者の健康食品の利用状況、店舗での販売の有無〉
回答者自身がサプリメントを利用しているか、という問に対し、73.1%の医師と 45.4%の薬剤師が「利用していない」と回答した。店舗でサプリメントの販売をしているか、という問いに対しては 92.4%の診療所でサプリメントの販売は行っていない一方、約 60%の薬局ではサプリメントの販売を行なっており、診療所と薬局で大きく異なる結果となった。
〈健康食品に関する相談を受ける頻度〉
43.4%の医師と 66.0%の薬剤師から健康被害の相談を受けたことがあるという回答を得た。相談件数は薬局のほうが多い傾向があるが、月に 10 回以上の相談を受ける医師、薬剤師はそれぞれ 5%未満であり、頻繁に相談を受ける医師、薬剤師は少ない傾向が見られた。
〈健康食品の健康被害に関する聞き取りの工夫〉
聞き取りの工夫を特に行なっていないと回答した医師は 95.7%、薬剤師が 87.1%であり、大多数を占めた。聞き取りに関してのフォーマットがあると回答した医師が 1.3%、薬剤師が 2.4%、業務手順として定めていると回答した医師が 1.7%、薬剤師が 5.9%であった。
〈健康被害の聞き取りと対処法〉
健康被害を聞き取った際にどう対処したか、という質問に対しては医師、薬剤師ともに、中止の勧告をしたという回答が最も多かった。
〈原因についての調査状況〉
健康被害の報告を受けて、製品や成分について調べたかどうかという問いに対して、医師より若干多く、薬剤師で、「調べた」という回答が得られた。メーカーの情報サイト、NMCD （Natural Medicine Comprehensive Database）を用いたという回答の他、食品に記載してある成分表、薬剤師会の DI で調べた、といった回答も見られた。
〈保健所への報告の有無と、報告しなかった理由〉
99.2%の医師は保健所へは報告していないと回答した。報告しなかった理由としては、報告するほどの被害でないと判断したから、という理由が最も多く、製品が原因と断定できなかった、という回答が次に多く認められた。一方、薬剤師においても、98.0%の薬剤師は保健所へは報告していないと回答した。報告しなかった理由としては製品が原因と断定できなかった、という理由が最も多く、報告するほどの被害でないと判断したから、という回答が次に多く認められた。他に、報告する場所があるとは知らなかった、との回答も得られた。
〈被害報告のあった健康食品〉
覚えていない、という回答が多く見られたが、成分名が示してあったものとして、グルコサミンやウコンという回答があった。
〈保健所へ報告の際、支障となると思われること〉

因果関係の判別が難しいという意見が71.5%の医師と、82.5%の薬剤師で認められた。次いで、どの程度の症状で報告すべきかわからないという意見が多く、他に、報告のマニュアルが無いので報告方法がわからない、といった意見が得られた。

〈報告手段として有用なもの〉

医師の63.9%、薬剤師の66.4%がFAXを選択した。その他に、電話、E-mailといった回答も多く見られた。

〈自由意見〉

医師、薬剤師ともに、健康食品の広告を規制すべき、健康被害報告をまとめたものを発信してほしい、報告用のフォーマットがほしいという意見の記載が認められた。

D. 考察

現在は健康食品の摂取に関連した健康被害相談の件数自体が少なく、情報を聴取する工夫が必要であることが示唆された。ほとんどの施設が聞き取りの工夫を行なっておらず、工夫による改善の可能性は高いと考えられる。

因果関係の判別の困難さが報告の支障になっていることに対しては、これまでに当研究室が開発した因果関係評価手法（*BMJ Open.* 5:e009038, 2015）の利用が有用であると考えられる。因果関係評価手法の利活用とともに、報告のフォーマットの作成やFAX等を利用した報告手段を診療所や薬局へと情報を伝達することで、保健所への報告状況の改善が見込まれる。

E. 結論

聴取の工夫や報告についての情報伝達と共に、因果関係評価手法の利活用を推進することで、保健所への報告にあたっての支障が解消され、保健所への報告状況が改善される可能性が示唆された。

F. 研究発表

1. 論文発表

1) Ide K, Yamada H, Kitagawa M, Kawasaki Y, Buno Y, Matsushita K, Kaji M, Fujimoto K, Waki M, Nakashima M, Umegaki K. Methods for estimating causal relationships of adverse events with dietary supplements. *BMJ Open.* 5(11):e009038, 2015

2) Ide K, Yamada H, Umegaki K, Mizuno K, Kawakami N, Hagiwara Y, Matsumoto M, Yoshida H, Kim K, Shiosaki E, Yokochi T, Harada K. Lymphocyte vitamin C level as potential biomarker for progression of Parkinson's disease. *Nutrition* 31:406-408, 2015.

3) Ide K. Yamada H, Clinical benefits of green tea consumption for cognitive dysfunction. *PharmaNutrition* 3(4): 136-145, 2015.

4) 山田浩. サプリメントの現状と臨床医の役割. Functional Food 9(2): 106-108, 2015.

2. 学会発表

1) 武野佑磨、井出和希、北川護、松下久美、加治正行、川崎洋平、梅垣敬三、山田浩. 健康食品の摂取に伴う健康被害報告の因果関係評価：架空事例による信頼性の検討. 第18回日本医薬品情報学会学術大会、岡山、2015年6月27-28日

2) 山田浩：シンポジウム「老化制御を目指した食品開発研究の現状」緑茶の高齢者における認知機能低下改善作用、第11回日本食品免疫学会学術大会東京、2015年10月15-16日

3) 山田浩：シンポジウム「医薬品と健康食品のエビデンスは同じか？」—健康食品のランダム化比較試験の例. 第36日本臨床薬理学会学術総会、新宿、2015年12月8-10日

4) 山田浩：シンポジウム「緑茶の機能性食品科学」緑茶の効用－臨床研究のエビデンス－、第13回 日本機能性食品医用学会総会、福岡、2015年12月11-12日

5) 山田浩：シンポジウム「食の安全性・機能性を支える科学の最前線」機能性食品の安全性・有用性評価法、日本薬学会年会. 横浜、2016年3月26-29日

G. 知的所有権の取得状況

特になし

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	巻号	ページ	出版年
Ide K, Yamada H, Kitagawa M, Kawasaki Y, Buno Y, Matsushita K, Kaji M, Fujimoto K, Waki M, Nakashima M, Umegaki K.	Methods for estimating causal relationships of adverse events with dietary supplements.	BMJ Open	5(11)	e009038	2015
Ide K, Yamada H, Umegaki K, Mizuno K, Kawakami N, Hagiwara Y, Matsumoto M, Yoshida H, Kim K, Shiosaki E, Yokochi T, Harada K.	Lymphocyte vitamin C level as potential biomarker for progression of Parkinson's disease.	Nutrition	31	406-408	2015
Ide K. Yamada H	Clinical benefits of green tea consumption for cognitive dysfunction.	PharmaNutrition	3(4)	136-145	2015
Chiba T, Sato Y, Suzuki S, Umegaki K.	Concomitant use of dietary supplements and medicines in patients due to miscommunication with physicians in Japan.	Nutrients	7	2947-60	2015
佐藤陽子、村田 美由貴、千葉剛、梅垣敬三	ワルファリン服用者におけるビタミンK摂取量の許容範囲に関する系統的レビュー	食品衛生学雑誌	56	157- 165	2015
千葉剛、佐藤陽子、鈴木祥菜、梅垣敬三	特定保健用食品と医薬品の併用者に関する実態調査	日本栄養・食糧学会誌	68	147-155	2015

BMJ Open

Methods for estimating causal relationships of adverse events with dietary supplements

Kazuki Ide,[1] Hiroshi Yamada,[1] Mamoru Kitagawa,[1] Yohei Kawasaki,[1] Yuma Buno,[1] Kumi Matsushita,[2] Masayuki Kaji,[3] Kazuko Fujimoto,[4] Masako Waki,[5] Mitsuyoshi Nakashima,[6] Keizo Umegaki[7]

To cite: Ide K, Yamada H, Kitagawa M, *et al*. Methods for estimating causal relationships of adverse events with dietary supplements. *BMJ Open* 2015;**5**:e009038. doi:10.1136/bmjopen-2015-009038

► Prepublication history for this paper is available online. To view these files please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2015-009038).

Received 10 June 2015
Revised 24 September 2015
Accepted 23 October 2015

For numbered affiliations see end of article.

Correspondence to
Professor Hiroshi Yamada;
hyamada@u-shizuoka-ken.ac.jp

ABSTRACT

Objective: Dietary supplement use has increased over past decades, resulting in reports of potentially serious adverse events. The aim of this study was to develop optimised methods to evaluate the causal relationships between adverse events and dietary supplements, and to test these methods using case reports.

Design: Causal relationship assessment using prospectively collected data.

Setting and participants: 4 dietary supplement experts, 4 pharmacists and 11 registered dietitians (5 men and 14 women) examined 200 case reports of suspected adverse events using the modified Naranjo scale and the modified Food and Drug Administration (FDA) algorithm.

Primary outcome measures: The distribution of evaluation results was analysed and inter-rater reliability was evaluated for the two modified methods employed using intraclass correlation coefficients (ICC) and Fleiss' κ.

Results: Using these two methods, most of the 200 case reports were categorised as 'lack of information' or 'possible' adverse events. Inter-rater reliability among entire assessors ratings for the two modified methods, based on ICC and Fleiss' κ, were classified as more than substantial (modified Naranjo scale: ICC (95% CI) 0.873 (0.850 to 0.895); Fleiss' κ (95% CI) 0.615 (0.615 to 0.615). Modified FDA algorithm: Fleiss' κ (95% CI) 0.622 (0.622 to 0.622).

Conclusions: These methods may help to assess the causal relationships between adverse events and dietary supplements. By conducting additional studies of these methods in different populations, researchers can expand the possibilities for the application of our methods.

Strengths and limitations of this study

- There is no optimised method for evaluating these adverse events.
- We developed two methods for assessing adverse events associated with dietary supplements and inter-rater reliability among entire assessors was classified as more than substantial.
- Our methods may be useful for assessing adverse events caused by dietary supplements in clinical settings.
- This simple and easy method for evaluating causal relationships can contribute to prompt issue evaluation, signal detection and regulatory updating.
- Additional studies with different populations are needed to expand the possibilities for application of our methods.

INTRODUCTION

The entire functional food market is estimated to be worth over US$80 billion.[1] This market reached US$32.5 billion in the USA in 2012,[2] with more than half of the adults reporting use of one or more dietary supplements. Sales of dietary supplements have also increased in Japan, with an estimated market size second only to that of the USA.[1] In fact, one study indicated that over 50% of the Japanese population consumes dietary supplements.[3] With the increased use of dietary supplements, a number of adverse events have been reported.[4–8] Some of these adverse events can lead to severe disability or death, so managing risk and safety is essential in order to protect consumers. Several legal systems have been developed to regulate labelling and manufacturing standards for dietary supplements, but there are no clear systems in place to detect and report adverse events.[9–11]

Evaluation of the causality of adverse events is essential in order to determine the risk and safety of supplements. It can also help with issue evaluation, signal detection and regulatory updating. Several methods exist for evaluating causality, including the Naranjo scale,[12 13] the Food and Drug Administration (FDA) algorithm,[14–16] the Kramer scale,[13 17] the Liverpool scale[18] and

the WHO scale.[19] However, these methods are primarily used to assess adverse events associated with medications. They are not optimised for application to dietary supplements. The information available from consumers taking dietary supplements differs from information provided by patients taking medications. Therefore, the development and optimisation of methods to evaluate the causal relationship between adverse events and dietary supplements is essential in order to improve the quality of risk management.

In the present study, we modified the Naranjo scale and the FDA algorithm and then used these to assess 200 case reports of suspected adverse reactions to dietary supplements. The main objective of this study was to test these modified methods using case reports.

METHODS

Study design

The Naranjo[12 13] scale and the FDA algorithm[14–16] were modified for use with dietary supplements. Two hundred case reports were randomly sampled from a database of adverse event reports associated with dietary supplements. Case reports in the database were based on consumers' voluntary reports through telephone calls to the consumer information centre in Japan and were not standardised for the evaluation of causal relationships. We recruited assessors from six institutions in Japan (University of Shizuoka, Keio University, Kikugawa General Hospital, Shizuoka City Shizuoka Hospital, Shizuoka City Public Health Center and Hamamatsu Institute of Clinical Pharmacology and Therapeutics) by announcement. Nineteen assessors (4 dietary supplement experts, 4 pharmacists and 11 registered dietitians; 5 men and 14 women) enrolled and evaluated the case reports by alternately using the modified Naranjo scale and the modified FDA algorithm. The characteristics of the 19 assessors are shown in table 1. Three dietary supplement experts worked at a general hospital and one worked at a university as a full professor. All four of the pharmacists worked at a general hospital. Four of the registered dietitians worked at a general hospital, and seven worked at a city healthcare centre. None of the assessors received any training in the use of the two scales, and they were not familiar with causal assessment of adverse drug reactions since earlier.

Table 1 Assessor characteristics

	Dietary supplement expert	Pharmacist	Registered dietitian
Number, n	4	4	11
Age, mean±SD	65.8±11.5	37.8±7.8	42.2±12.4
Sex, n (%)			
Men	1 (25)	3 (75)	1 (9)
Women	3 (75)	1 (25)	10 (91)
Career length, mean years±SD	33.5±2.4	8.6±13.8	13.9±17.4

Assessment scale design

Modified Naranjo scale

The modified Naranjo scale is shown in figure 1. The phrase 'drug' in the Naranjo scale was changed to 'dietary supplement'. The section in question 3 of the Naranjo scale pertaining to a specific antagonist was deleted. Questions regarding placebo and blood (or other fluid) concentrations were excluded. In addition to these changes, the scoring for questions pertaining to readministration and confirmation by objective evidence was changed by adding one point for positive answers to the original version of the Naranjo scale. The adverse event reports were assigned to a probability category using the total scores as follows: ≥9 highly probable, 5–8 probable, 3–4 highly possible, 1–2 possible, ≤0 unlikely. Case reports lacking information about time relationships were excluded and categorised as 'lack of information'.

Modified FDA algorithm

Details of the FDA algorithm have been described previously.[16] The modified FDA algorithm is shown in figure 2. There was limited information included in the dietary supplement case reports, so the number of options for questions was changed from 2 to 3: 'Yes', 'No' and 'Don't know'. The scale was structured with 4 primary questions and 5 branch questions. The contents of the main questions were as follows: (1) the temporal relationship; (2) changes in symptoms due to the dietary supplement being discontinued; (3) rechallenges and (4) objective evidence from laboratory tests such as a drug-induced lymphocyte stimulation test or patch test. Each of these questions had branch questions relating to: (1) existing clinical conditions; (2) objective evidence from laboratory tests such as a drug-induced lymphocyte stimulation test or patch test and (3) previous experiences of adverse events after taking the same or similar (eg, including the same ingredient) dietary supplement. Adverse event reports were assigned to one of the following probability categories on the basis of the answers to these questions: lack of information, unlikely, possible, highly possible, probable and highly probable.

Statistical analysis

In order to quantify the level of agreement in the modified Naranjo scale, intraclass correlation coefficients (ICCs) with a 95% CI were calculated using the methods described by Shrout and Fleiss.[20] ICCs were interpreted according to the following criteria:<0.40, poor agreement; 0.40–0.75, moderate agreement and >0.75, excellent agreement.[21]

Inter-rater (multirater) reliability for the modified Naranjo scale and the modified FDA algorithm was analysed using Fleiss' κ with a SE.[22] Fleiss' κ values were also calculated for each question of the modified Naranjo

Figure 1 Modified Naranjo scale.

No	Question	Yes	No	Do Not Know
1	Are there any notification about the reaction on the label or package insert of the dietary supplement?	+1	0	0
2	Did the adverse event appear after suspected dietary supplement intake?	+2	-1	0
3	Did the adverse reaction improve when the suspected dietary supplement was discontinued?	+2	0	0
4	Did the adverse event reappear when the dietary supplements re-intake?	+3	-1	0
5	Are there alternative causes (other than the dietary supplement) that could on their own have caused the reaction?	-1	+2	0
6	Was the reaction more severe when the dose was increased or less severe when the dose was decreased?	+1	0	0
7	Did the consumer have a similar reaction to the same or similar dietary supplements in any previous exposure?	+1	0	0
8	Was the adverse event confirmed by any objective evidence?	+2	0	0

scale. The 95% CI of Fleiss' κ was calculated from its SE. Fleiss' κ values were interpreted according to the criteria defined by Landis and Koch[23]: −1.00, total disagreement; 0.00, no agreement; 0.01–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60 moderate agreement; 0.61–0.80 substantial agreement; 0.81–0.99 almost perfect agreement and 1.00, perfect agreement. All statistical analyses were performed using SAS V.9.4 for Windows (SAS Institute Inc, Cary, North Carolina, USA).

RESULTS

The modified Naranjo scale and the modified FDA algorithm are shown in figures 1 and 2. All assessors evaluated 200 case reports using the modified Naranjo scale and the modified FDA algorithm. No results were missing from the case report evaluations. The distribution of evaluation results is shown in figure 3A, B. These case reports were based on voluntary consumer reports, included incomplete reporting and were not standardised for the evaluation of causal relationships. Most of the 200 case reports were categorised as 'lack of information' or 'possible'. The median (range) of cases in 'lack of information' using the modified Naranjo scale was 64 (8-143) and the corresponding values using the modified FDA scale were 64 (8-142) cases. The 'possible' category included a median (range) of 88 (19-136) cases using the modified Naranjo scale and 90 (17-138) cases using the modified FDA scale. The information on dosage, previous similar events and objective evidence was particularly poorly reported in these case reports. A large proportion of the cases were mild. Skin symptoms such as pruritus (n=56) and gastrointestinal symptoms such as abdominal discomfort (n=62) were the most common. However, two serious adverse events related to hepatic dysfunction were reported. In one serious case, a woman started to take a dietary supplement for weight loss. Two weeks after commencing this treatment, her health deteriorated and she presented at a general hospital. Laboratory analyses revealed abnormal hepatic enzyme results and she was diagnosed with liver dysfunction. This condition resolved after over 2 weeks of

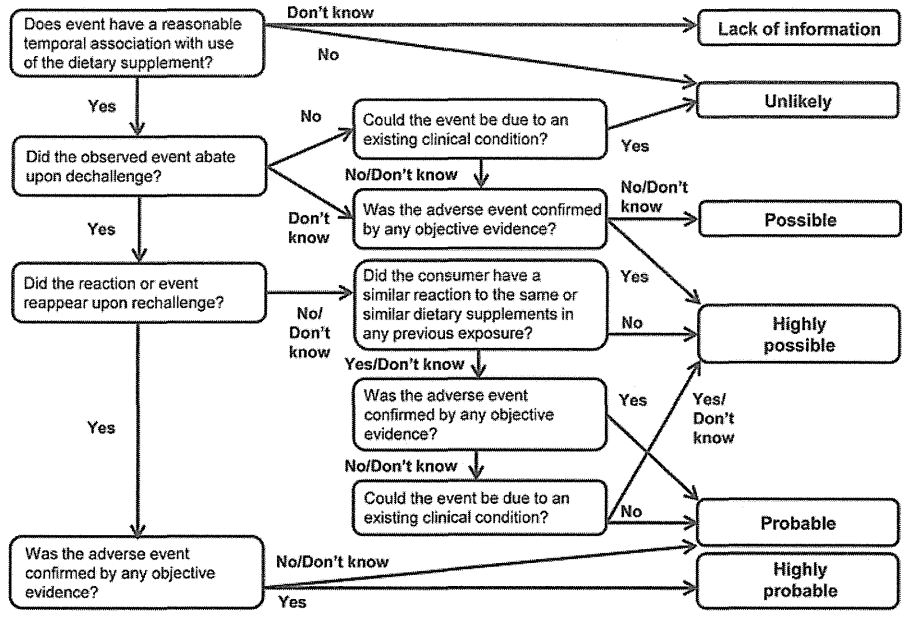


Figure 2 Modified Food and Drug Administration (FDA) algorithm.

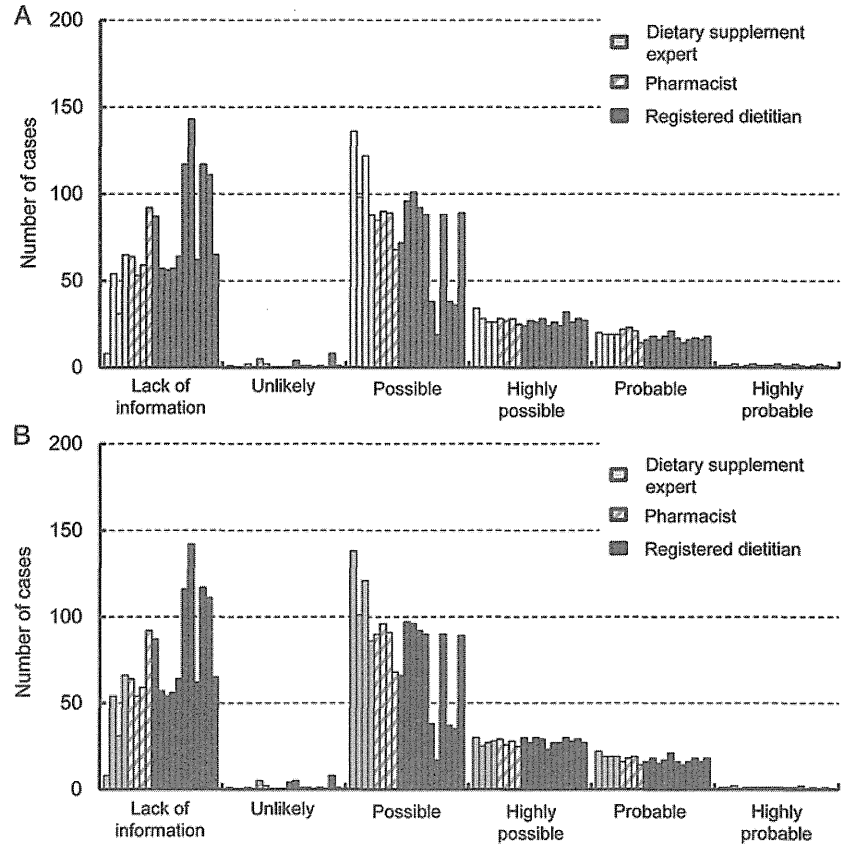


Figure 3 (A) Distribution of results for the modified Naranjo scale. (B) Distribution of results for the modified Food and Drug Administration (FDA) algorithm.

hospitalisation. The attending doctor considered that the patient's dietary supplement had caused her liver dysfunction. In another case, a woman had been taking a dietary supplement for weight control for several months and had experienced fatigue for several weeks. She presented at a general hospital, where laboratory analyses revealed abnormal hepatic enzyme results and she was diagnosed with hepatitis. Her attending doctor considered that this was due to the dietary supplement. The patient's hepatitis improved after around 2 weeks' hospitalisation.

Modified Naranjo scale

The ICCs and Fleiss' κ coefficient (Fleiss' κ) values for the modified Naranjo scale are shown in table 2. The ICCs (95% CI) for each assessor group were as follows: dietary supplement experts, 0.865 (0.836 to 0.891); pharmacists, 0.890 (0.865 to 0.911) and registered dietitians, 0.882 (0.859 to 0.903). For the entire group of assessors, this value was 0.873 (0.850 to 0.895). Fleiss' κ values (95% CI) for each assessor group were as follows: dietary supplement experts, 0.598 (0.596 to 0.599); pharmacists, 0.791 (0.790 to 0.792) and registered dietitians, 0.610 (0.609 to 0.610). For the entire group of assessors, this value was 0.615 (0.615 to 0.615). The levels of agreement based on the ICCs for each assessor group and all assessors combined were excellent. Inter-rater (multirater) reliability classifications based on Fleiss' κ were as follows: fair agreement among dietary supplement experts and substantial agreement among pharmacists, registered dietitians and the entire group as a whole.

Fleiss' κ values (95% CI) for each question of the modified Naranjo scale were as follows: item 1 (product labelling), 0.048 (−0.169 to 0.264); item 2 (temporal relationship), 0.530 (0.530 to 0.531); item 3 (changes in adverse event after discontinuation), 0.944 (0.943 to 0.945); item 4 (rechallenges), 0.861 (0.857 to 0.866); item 5 (other factors related to the adverse event), 0.585

Table 2 ICC and Fleiss' κ coefficient values for the modified Naranjo scale and the modified FDA algorithm

	Modified Naranjo scale		Modified FDA algorithm
	κ Coefficient (95% CI)	ICC (95% CI)	κ Coefficient (95% CI)
Dietary supplement expert (n=4)	0.598 (0.596 to 0.599)	0.865 (0.836 to 0.891)	0.596 (0.594 to 0.598)
Pharmacist (n=4)	0.791 (0.790 to 0.792)	0.890 (0.865 to 0.911)	0.780 (0.779 to 0.781)
Registered dietitian (n=11)	0.610 (0.609 to 0.610)	0.882 (0.859 to 0.903)	0.624 (0.623 to 0.624)
Total (n=19)	0.615 (0.615 to 0.615)	0.873 (0.850 to 0.895)	0.622 (0.622 to 0.622)

FDA, Food and Drug Administration; ICC, intraclass correlation coefficient.

(0.584 to 0.585); item 6 (dose dependency), 0.797 (0.754 to 0.840); item 7 (adverse event history), 0.057 (0.022 to 0.093) and item 8 (objective evidence from laboratory tests), 0.561 (0.519 to 0.603). Items 1 and 7 showed the two lowest levels of agreement.

Modified FDA algorithm

Fleiss' κ values for the modified FDA algorithm are shown in table 2. Fleiss' κ values (95% CI) for each assessor group were as follows: dietary supplement experts, 0.596 (0.594 to 0.598); pharmacists, 0.780 (0.779 to 0.781) and registered dietitians, 0.624 (0.623 to 0.624). For all 19 assessors, this value was 0.622 (0.622 to 0.622). Inter-rater (multirater) reliability based on Fleiss' κ values were as follows: fair agreement among dietary supplement experts; substantial agreement among pharmacists, registered dietitians and the entire group of assessors as a whole.

DISCUSSION

In this study, we modified the Naranjo scale and the FDA algorithm and used them to evaluate case reports of adverse reactions to dietary supplements. These reports were assessed by dietary supplement experts, pharmacists and registered dietitians.

Agreement levels for the Naranjo scale, based on ICCs for each individual group and the assessor group as a whole, were classified as 'excellent'. Fleiss' κ values for each assessor group and for the group as a whole also demonstrated more than fair agreement. These results indicated that the modified Naranjo scale would be useful for evaluating the causal relationships between adverse events and dietary supplements. It may also have broad utility among different professions. The only concerns were items 1 and 7 (product labelling and adverse event history, respectively), which produced the two lowest levels of agreement. To remedy this, assessors might easily obtain the information from consumers as they are reporting the adverse events. Revising these two items and also recording consumers' reports as they occur may improve the inter-rater (multirater) reliability and usability of the modified Naranjo scale.

The modified FDA algorithm showed more than fair agreement between each assessor group and within the entire group. Like the Naranjo scale, it has broad utility and would be useful for assessing the causality of adverse events.

For both methods, the inter-rater (multirater) reliability ratings determined using ICCs and Fleiss' κ analyses showed more than substantial agreement in the entire group of assessors. In fact, the Fleiss' κ values were very similar (0.615 for the modified Naranjo scale vs 0.622 for the modified FDA algorithm). Between them, scientists could select the one that best suits their purpose.

A large proportion of the 200 cases assessed in this study reported mild symptoms, although two serious cases with hepatic dysfunction were included. Although mild symptoms are not life-threatening, they do affect the quality of life. Therefore, analysis of causal relationships and the provision of information can improve the safety of dietary supplement usage. The number of serious adverse events was limited but these can lead to severe disability; the analysis of causality using this method can lead to prompt diagnosis and treatment, as well as regulatory actions.

There were several limitations to this study. The main limitation was the distribution of evaluation results. For both evaluation methods, most of the 200 case reports were categorised as 'lack of information' or 'possible'. This may reflect the limited information included in the case reports used in this study. Case reports were based on voluntary consumer telephone calls and were not structured to facilitate evaluation of causal relationships. This might have affected the inter-rater (multirater) reliability ratings. In fact, most of the disagreements among assessors related to classification as either 'lack of information' or 'possible', while there was fairly good agreement concerning 'highly possible', 'probable' and 'highly probable' cases. This may be due to the evaluation based on speculation of each assessor in the cases categorised as 'lack of information' or 'possible'. Structured or semi-structured standardised interviews of consumers can improve the quality of information in case reports. When designing a structured or semistructured interview form, information on dosage, previous similar events and objective evidence should be requested, in addition to the essential information regarding temporal association and discontinuation. Even in the cases categorised as 'probable', some of these items of information were absent. For example, a man started to take a dietary supplement for health enhancement, and then developed oral inflammation. After discontinuation of the supplement, his oral inflammation resolved. When he started to take the dietary supplement again, oral inflammation recurred and he then stopped taking the supplement. This case included information on temporal association, discontinuation and rechallenge, but lacked information on dosage, previous similar events and objective evidence. Validity of the methods may also be a limitation. We estimated inter-rater (multirater) reliability using ICCs and Fleiss' κ. However, these methods were not validated. Future studies could validate these methods in different populations in order to address this limitation and expand the potential for application of our methods in other clinical and regulatory settings. For example, medical institutions and regulatory agencies might use these modified methods to screen for adverse effects associated with dietary supplements, which may accelerate the detection of harmful events.

The FDA currently operates the Safety Reporting Portal[24] for organisations, professionals and consumers. The Safety Reporting Portal is the electronic version of MedWatch 3500, 3500A and 3500B,[25] which are voluntary reporting forms for adverse events, tailored to dietary supplements. However, researchers point out that these data sets contain many incomplete reports. Other national or local health departments are often

the first to detect harm,[9] because these forms are detailed and possibly too complicated for people to use.[26] Combining a screening tool with detailed surveillance will make the reporting system more user friendly. This may promote voluntary reporting and lead to more rapid detection of harmful events.

In summary, we present a modified Naranjo scale and a modified FDA algorithm that may be used to assess the causal relationships between adverse events and dietary supplements. These tools might also be used by regulatory agencies to screen for adverse supplement events, but additional studies are needed to expand the possibilities for application of our methods.

Author affiliations
[1]Department of Drug Evaluation & Informatics, Graduate School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan
[2]Kikugawa General Hospital, Shizuoka, Japan
[3]Shizuoka City Public Health Center, Shizuoka, Japan
[4]Faculty of Pharmacy, Keio University, Tokyo, Japan
[5]Shizuoka City Shizuoka Hospital, Shizuoka, Japan
[6]Hamamatsu Institute of Clinical Pharmacology & Therapeutics, Shizuoka, Japan
[7]Information Center, National Institutes of Biomedical Innovation, Health and Nutrition, Tokyo, Japan

Acknowledgements The authors would like to acknowledge the assessors in this study.

Contributors KI, HY and M Kitagawa designed the study. KI, HY, YB, M Kitagawa, KM, M Kaji and KU performed the research and collected the data. YK, KI and YB analysed the data. KI, HY and YK wrote the manuscript. All the authors reviewed and approved the contents of the manuscript.

Funding This work was supported in part by a grant from the Japanese Ministry of Health, Labour and Welfare (No. 24220501 to HY and KU), and a grant from the Japan Society for the Promotion of Science (JSPS) through the Grant-in-Aid for JSPS Fellows (No.15J10190 to KI).

Competing interests None declared.

Ethics approval This study protocol was approved by the ethics committee of the University of Shizuoka (No. 26-6, 2014).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Additional data can be accessed via the Dryad data repository at http://datadryad.org/ with the doi:10.5061/dryad.5ts48.

REFERENCES

1. Vergari F, Tibuzzi A, Basile G. An overview of the functional food market: from marketing issues and commercial players to future demand from life in space. *Adv Exp Med Biol* 2010;698:308–21.
2. Garcia-Cazarin ML, Wambogo EA, Regan KS, *et al.* Dietary supplement research portfolio at the NIH, 2009–2011. *J Nutr* 2014;144:414–18.
3. Imai T, Nakamura M, Ando F, *et al.* Dietary supplement use by community-living population in Japan: Data from the National Institute for Longevity Sciences Longitudinal Study of Aging (NILS-LSA). *J Epidemiol* 2006;16:249–60.
4. Stickel F, Shouval D. Hepatotoxicity of herbal and dietary supplements: an update. *Arch Toxicol* 2015;89:851–65.
5. Patel DN, Low WL, Tan LL, *et al.* Adverse events associated with the use of complementary medicine and health supplements: An analysis of reports in the Singapore Pharmacovigilance database from 1998 to 2009. *Clin Toxicol* 2012;50:481–9.
6. Haller C, Kearney T, Bent S, *et al.* Dietary supplement adverse events: report of a one-year poison center surveillance project. *J Med Toxicol* 2008;4:84–92.
7. Timbo BB, Ross MP, McCarthy PV, *et al.* Dietary supplements in a national survey: prevalence of use and reports of adverse events. *J Am Diet Assoc* 2006;106:1966–74.
8. Pittler MH, Schmidt K, Ernst E. Adverse events of herbal food supplements for body weight reduction: systematic review. *Obes Rev* 2005;6:93–111.
9. Cohen PA. Hazards of hindsight—monitoring the safety of nutritional supplements. *N Engl J Med* 2014;370:1277–80.
10. Frankos VH, Street DA, O'Neill RK. FDA regulation of dietary supplements and requirements regarding adverse event reporting. *Clin Pharmacol Ther* 2010;87:239–44.
11. Chiba T, Sato Y, Nakanishi T, *et al.* Inappropriate usage of dietary supplements in patients by miscommunication with physicians in Japan. *Nutrients* 2014;6:5392–404.
12. Naranjo CA, Busto U, Sellers EM, *et al.* A method for estimating the probability of adverse drug reactions. *Clin Pharmacol Ther* 1981;30:239–45.
13. Busto U, Naranjo CA, Sellers EM. Comparison of two recently published algorithms for assessing the probability of adverse drug reactions. *Br J Clin Pharmacol* 1982;13:223–7.
14. Irey NS. Adverse drug reactions and death. A review of 827 cases. *JAMA* 1976;236:575–8.
15. Karch FE, Lasagna L. Toward the operational identification of adverse drug reactions. *Clin Pharmacol Ther* 1977;21:247–54.
16. Jones JK. Adverse drug reactions in the community health setting: approaches to recognizing, counseling, and reporting. *Fam Community Health* 1982;5:58–67.
17. Kramer MS, Leventhal JM, Hutchinson TA, *et al.* An algorithm for the operational assessment of adverse drug reactions. I. Background, description, and instructions for use. *JAMA* 1979;242:623–32.
18. Gallagher RM, Kirkham JJ, Mason JR, *et al.* Development and inter-rater reliability of the Liverpool adverse drug reaction causality assessment tool. *PLoS ONE* 2011;6:e28096.
19. World Health Organization. WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. http://apps.who.int/medicinedocs/documents/s7148e/s7148e.pdf (accessed 5 Jun 2015).
20. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychol Bull* 1979;86:420–8.
21. Rosner B. *Fundamentals of biostatistics.* Pacific Grove, CA: Duxbury Press, 2005.
22. Fleiss JL. Measuring nominal scale agreement among many raters. *Psychol Bull* 1971;76:378.
23. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;33:159–74.
24. Safety Reporting Portal. https://www.safetyreporting.hhs.gov/ (accessed 5 Jun 2015).
25. Kessler DA. Introducing MEDWatch. A new approach to reporting medication and device adverse effects and product problems. *JAMA* 1993;269:2765–8.
26. Getz KA, Stergiopoulos S, Kaitin KI. Evaluating the completeness and accuracy of MedWatch data. *Am J Ther* 2014;21:442–6.

Nutrition 31 (2015) 406–408

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Brief report

Lymphocyte vitamin C levels as potential biomarker for progression of Parkinson's disease

Kazuki Ide M.Sc. [a], Hiroshi Yamada M.D., Ph.D. [a,b,*], Keizo Umegaki Ph.D. [c], Katsuki Mizuno B.A. [a], Nobuko Kawakami M.D. [b], Yuka Hagiwara M.D. [b], Mizuki Matsumoto M.D. [b], Hidefumi Yoshida M.D. [b], Kang Kim M.D. [b], Emi Shiosaki B.A. [d], Tsunehiro Yokochi B.A. [d], Kiyoshi Harada M.D., Ph.D. [b]

[a] *Department of Drug Evaluation and Informatics, Graduate School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan*
[b] *Department of Neurology, Shizuoka General Hospital, Shizuoka, Japan*
[c] *National Institute of Health and Nutrition, Tokyo, Japan*
[d] *Department of Clinical Laboratory Medicine, Shizuoka General Hospital, Shizuoka, Japan*

ARTICLE INFO

Article history:
Received 2 May 2014
Accepted 2 August 2014

Keywords:
Parkinson's disease
Clinical assessment
Biomarkers
Oxidative stress
Vitamin C

ABSTRACT

Objectives: Vitamin C is a major antioxidant and also is known as a neuromodulator in dopaminergic neurons. The aim of this study was to investigate the association between lymphocyte and plasma vitamin C levels in various stages of Parkinson's disease (PD).
Methods: Sixty-two individuals with PD (age 71 ± 8.8 y [mean ± SD]) being treated at Shizuoka General Hospital from December 2007 to August 2013 were consecutively recruited. PD severity was classified using the Hoehn-Yahr scale for staging PD. Fasting blood samples were collected, and plasma and lymphocyte vitamin C levels were measured. The association between PD severity and vitamin C levels was estimated by ordinal logistic regression with confounding variables.
Results: The distribution of Hoehn-Yahr stages in patients was as follows: stage I, 7; II, 28; III, 16; and IV, 11. Lymphocyte vitamin C levels in patients with severe PD were significantly lower (odds ratio [OR], 0.87; 95% confidence interval [CI], 0.80–0.97; $P < 0.01$) compared with those at less severe stages. Plasma vitamin C levels also tended to be lower in patients with severe PD; however, this was not significant (OR, 0.98; 95% CI, 0.96–1.00; $P = 0.09$).
Conclusions: Our findings suggest that lymphocyte vitamin C levels in the peripheral blood may be a potentially useful biomarker for the progression of PD.

Introduction

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder in the world [1]. The pathologic hallmarks of PD include the loss of dopaminergic neurons in the substantia nigra pars compacta and the presence of Lewy bodies [1,2]. Increased oxidative stress in the brain has been hypothesized to contribute to the neurodegeneration and neuronal dysfunction in PD [3–5]. An increase of oxidative stress is caused by an imbalance between reactive oxygen species and antioxidants. There are several markers of oxidative stress, including damaged DNA bases (e.g., 8-hydroxydeoxyguanosine), oxidized lipids, and decreased antioxidants [6–8]. However, the number of these biomarkers used in clinical practice is limited, and the importance of peripheral oxidative stress markers in PD has not yet been clarified [6,9].

Vitamin C is a major antioxidant detected in various components of the peripheral blood. In lymphocytes, vitamin C levels are 80 to 100 times higher than in plasma [10]; moreover, these levels do not seem to be affected by transient dietary changes and circadian rhythms [11]. We previously reported lower lymphocyte vitamin C levels in individuals with type 2 diabetes mellitus compared with healthy controls [12]. Interestingly, this form of diabetes is associated with oxidative stress, suggesting the possibility that vitamin C levels in the peripheral blood could serve as a biomarker of oxidative stress in various diseases.

H. Yamada received a grant from the Japanese Ministry of Health, Labor, and Welfare (No. 242 20 501). KU received a grant from the Japanese Ministry of Health, Labor, and Welfare (No. 242 20 501). H. Yamada, KI, KU, and KH conceived of and designed the study. KM, NK, YH, MM, H. Yoshida, KK, ES, TY, and KH acquired the data. KI, H. Yamada, KU, and KM analyzed and interpreted the data. KI, H. Yamada drafted the manuscript.
* Corresponding author. Tel./fax: +81 54 264 5762.
E-mail address: hyamada@u-shizuoka-ken.ac.jp (H. Yamada).

0899-9007/$ - see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nut.2014.08.001

Table 1
Clinical characteristics and plasma/lymphocyte vitamin C levels

	Hoehn-Yahr scale stages				*P*-value*
	Stage I	Stage II	Stage III	Stage IV	
Clinical characteristics					
n	7	28	16	11	
Sex, n (%)					
Men	1 (14.3)	15 (53.6)	9 (56.3)	6 (54.5)	0.26†
Women	6 (85.7)	13 (46.4)	7 (43.8)	5 (45.5)	
Age, mean ± SD	73.0 ± 7.9	68.6 ± 9.0	70.5 ± 9.4	76.2 ± 5.7	0.09‡
Duration of PD, mean ± SD	1.4 ± 0.8	5.4 ± 5.9	7.1 ± 5.0	8.7 ± 4.8	0.03‡
BMI, mean ± SD	21.8 ± 2.5	21.0 ± 2.9	23.5 ± 3.3	22.4 ± 3.6	0.09‡
Smoking, n (%)	0 (0)	1 (3.6)	2 (12.5)	1 (9.1)	0.63†
Alcohol use, n (%)	3 (42.9)	6 (21.4)	5 (31.3)	0 (0)	0.09†
Health food, n (%)	2 (28.6)	8 (28.6)	3 (18.8)	4 (36.4)	0.80†
Vitamin C levels					
Plasma (μmol/L)	44.1 ± 25.0	43.5 ± 20.8	33.6 ± 17.1	30.0 ± 16.9	0.16‡
Lymphocyte (nmol/mg protein)	19.5 ± 3.7	19.3 ± 6.5	13.7 ± 3.6	14.5 ± 2.6	0.02‡
Multivariate analysis		OR (95% CI)			*P*-value*
Plasma vitamin C		0.80 (0.63–1.00)			0.09§
Age		1.03 (0.97–1.09)			0.29§
BMI		1.13 (0.97–1.32)			0.11§
Alcohol use		0.45 (0.14–1.43)			0.18§
Lymphocyte vitamin C		0.88 (0.79–0.97)			0.008§
Age		1.03 (0.98–1.10)			0.24§
BMI		1.12 (0.96–1.31)			0.15§
Alcohol use		0.045 (0.14–1.49)			0.19§

BMI, body mass index; PD, Parkinson's disease
* *P*-values were calculated using the following statistical analysis.
† Fisher's exact test.
‡ One-way analysis of variance.
§ Ordinal logistic regression.

Vitamin C also acts as an antioxidant in the brain and also is a known neuromodulator in dopaminergic and glutamatergic neurons. The relationship between vitamin C and dopaminergic function has been shown in vitro and in vivo [13–15]. Vitamin C also has been shown to protect neurons from glutamatergic neurotoxicity [16]. Several molecules have antioxidant properties similar to vitamin C, such as vitamin E and β-carotene. However, fewer experimental studies have been carried out on these molecules than on vitamin C in relation to neuromodulation in dopaminergic and glutamatergic neurons. In the present study, we investigated the association between plasma and lymphocyte vitamin C levels and PD severity.

Materials and methods

Participants

Sixty-two individuals with PD (age 71 ± 8.8 y [mean ± SD], age range 42–89 y; 31 men; 31 women) who were being treated at the Shizuoka General Hospital were consecutively enrolled in this study from December 2007 to August 2013. The patients were diagnosed with PD according to the criteria of Ward and Gibb, and the severity was classified based on Hoehn-Yahr stages. Individuals with secondary parkinsonisms, such as vascular and drug-induced parkinsonism, were excluded.

Written informed consent was obtained from all participants before enrollment. The study protocol was approved by the ethics committee of the University of Shizuoka (No. 19–17), Shizuoka General Hospital (No. 19–20) and conducted in accordance with the Declaration of Helsinki.

Study design

The following clinical characteristics were recorded: sex, age, duration of PD, body mass index (BMI), smoking habit, alcohol use, and health food consumption. Health food consumption was the consumption of vitamin and other antioxidant supplements. Blood samples were collected from patients under fasting conditions via venipuncture. Plasma and lymphocyte vitamin C levels were measured with the high-performance liquid chromatography with the electrochemical detector (HPLC-ECD) method.

Sample preparation and quantification of plasma and lymphocyte vitamin C

Plasma and lymphocytes were separated by Ficoll-Hypaque gradient centrifugation and then immediately treated with metaphosphoric acid (final 5% wt/wt) to stabilize vitamin C. All sample preparation was performed on ice within a 2-h time window to obtain reliable data, and samples were stored at −80°C until analysis. Vitamin C levels were measured by the HPLC-ECD method, and all samples were treated in the same manner [12,17].

Statistical analysis

Fisher's exact test was performed to make categorical comparisons of clinical characteristics. Differences in the mean values of continuous measurements among Hoehn-Yahr stages were tested by one-way analysis of variance. Linear correlations between plasma and lymphocyte vitamin C levels were estimated by Spearman's rank correlation (ρ). Clinical characteristics with a $P < 0.15$ were considered potential confounding variables. They were considered for multivariate analysis, and ordinal logistic regression was used to analyze the difference in plasma and lymphocyte vitamin C levels. Statistical significance was set at $P < 0.05$ and all statistical procedures were performed with the Statistical Package for the Social Sciences version 21.0 for Windows (IBM Corp., Armonk, NY, USA).

Results

Participants' clinical characteristics are presented in Table 1 with the distribution of Hoehn-Yahr stages as follows: stage I, 7; stage II, 28; stage III, 16; stage IV, 11. Of the clinical characteristics, age ($P = 0.05$), BMI ($P = 0.09$), and alcohol use ($P = 0.09$) were different in the stages of PD, and considered as confounding variables in the ordinal logistic regression analysis. Duration of PD also differed, but was not added to the regression because of collinearity with lymphocyte vitamin C levels ($P = 0.001$).

All blood samples obtained from the 62 participants were analyzed (Table 1) and plasma vitamin C levels at each PD stage were determined as follows (all mean ± SD, μmol/L): stage I, 44.5 ± 25.0; stage II, 43.5 ± 20.8; stage III, 33.6 ± 17.7; stage IV,

30.0 ± 16.9 ($P = 0.16$). In lymphocytes, vitamin C levels for each stage of PD were determined as follows (all mean ± SD, nmol/mg protein): stage I, 19.5 ± 3.7; stage II, 19.3 ± 6.5; stage III, 13.7 ± 3.5; stage IV; 14.5 ± 2.7 ($P = 0.02$). A significant linear correlation was found between plasma and lymphocyte vitamin C levels ($\rho = 0.45$, $P < 0.001$).

In the multivariate analysis, lymphocyte vitamin C levels were significantly lower in individuals with severe PD (odds ratio [OR], 0.87; 95% confidence interval [CI], 0.80–0.97; $P < 0.008$) compared with those at less severe stages of the disease. Plasma vitamin C levels also tended to be lower in patients at less severe stages of PD; however, this finding was not significant (OR, 0.98; 95% CI, 0.96–1.00; $P = 0.09$).

Discussion

In this study, we demonstrated that lymphocyte vitamin C levels were significantly lower in patients with severe PD, and that there was a linear correlation between plasma and lymphocyte vitamin C levels.

Correlations between vitamin C levels in the cerebrospinal fluid and peripheral blood have been reported [18]; we collected peripheral blood samples from participants with PD who were in fasting state. However, significant differences among PD stages were only observed in lymphocyte vitamin C levels. In general, lymphocyte vitamin C levels are much higher and more stable than plasma vitamin C levels. A decrease in the function of respiratory chain enzyme, which is related to free radical production modulation, in lymphocytes in patients with PD has been reported [19]. A link between an impairment of respiratory chain enzyme complex III assembly and an increase in free radical production also has been identified [20]. These properties may be affected by the difference in changes between plasma and lymphocyte vitamin C levels. Therefore, lymphocyte vitamin C levels may accurately reflect the progression of PD.

Lymphocyte vitamin C assays also have advantages for clinical use. Compared with other components of blood cells, lymphocyte assay is a simple method, and suitable for measuring vitamin C levels. Vitamin C is notoriously unstable; it decreased in a few hours at 4°C, even when the plasma was treated with methanol/EDTA [17]. Therefore, preparation time for this assay is important so that accurate results can be obtained.

Conclusions

Our results suggest that lymphocyte vitamin C levels could serve as a potentially useful biomarker of PD progression. Furthermore, monitoring lymphocyte vitamin C levels may contribute to improved diagnostic accuracy and could prove useful in the selection of effective therapeutic approaches for various stages of PD. However, further investigations including longitudinal studies on the relationship between changes in lymphocyte vitamin C levels and the progression of PD are required before it can be applied to clinical practice.

Acknowledgment

The authors gratefully acknowledge the participants in this study and the medical staff at the Shizuoka General Hospital.

References

[1] Jellinger KA. Neuropathology of sporadic Parkinson's disease: evaluation and changes of concepts. Mov Disord 2012;27:8–30.
[2] Halliday GM, McCann H. The progression of pathology in Parkinson's disease. Ann N Y Acad Sci 2010;1184:188–95.
[3] Yürekli VA, Gürler S, Nazıroğlu M, Uğuz AC, Koyuncuoğlu HR. Zonisamide attenuates MPP+-induced oxidative toxicity through modulation of Ca2+ signaling and caspase-3 activity in neuronal PC12 cells. Cell Mol Neurobiol 2013;33:205–12.
[4] Kenae PC, Kurzawa M, Blain PG, Morris CM. Mitochondrial dysfunction in Parkinson's disease. Parkinson's Disease 2011. Available at: http://dx.doi.org/10.4061/2011/716871. Accessed 2014 Sep 4.
[5] Hwang O. Role of oxiadtive stress in Parkinson's disease. Exp Neurobiol 2013;22:11–7.
[6] Sharma S, Moon CS, Khogali A, Haidous A, Chabenne A, Ojo C, et al. Biomarkers in Parkinson's disease (recent update). Neurochem Int 2013;63:201–29.
[7] Sato S, Mizuno Y, Hattori N. Urinary 8-hydroxydeoxyguanosine levels as a biomarker for progression of Parkinson disease. Neurology 2005;64:1081–3.
[8] Chen CM, Liu JL, Wu YR, Chen YC, Cheng HS, Cheng ML, et al. Increased oxidative damage in peripheral blood correlates with severity of Parkinson's disease. Neurobiol Dis 2009;33:429–35.
[9] King D, Playfer JR, Roberts NB. Concentrations of vitamins A, C and E in elderly patients with Parkinson's disease. Postgrad Med J 1992;68:634–7.
[10] Evans RM, Currie L, Campbell A. The distribution of ascorbic acid between various cellular components of blood, in normal individuals, and its relation to the plasma concentration. Br J Nutr 1982;47:473–82.
[11] Wilson CW. Clinical pharmacologic aspects of ascorbic acid. Ann N Y Acad Sci 1975;258:355–76.
[12] Yamada H, Yamada K, Waki M, Umegaki K. Lymphocyte and plasma vitamin C levels in type 2 diabetic patients with and without diabetes complications. Diabetes Care 2004;27:2491–2.
[13] Grunewald RA. Ascorbic acid in the brain. Brain Res Rev 1993;18:123–33.
[14] Ballaz S, Morales I, Rodriguez M, Obeso JA. Ascorbate prevents cell death from prolonged exposure to glutamate in an in vitro model of human dopaminergic neurons. J Neurosci Res 2013;91:1609–17.
[15] Bagga V, Dunnett SB, Fricker-Gates RA. Ascorbic acid increases the number of dopamine neurons in vitro and in transplants to the 6-OHDA-lesioned rat brain. Cell Transplant 2008;17:763–73.
[16] Harrison FE, May JM. Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2. Free Radic Biol Med 2009;46:719–30.
[17] Umegaki K, Inoue K, Takeuchi N, Higuchi M. Improved method for the analysis of ascorbic acid in plasma by high-performance liquid chromatography with electrochemical detection. J Nutr Sci Vitaminol (Tokyo) 1994;40:73–9.
[18] Bowman GL, Dodge H, Frei B, Calabrese C, Oken BS, Kaye JA, et al. Ascorbic acid and rates of cognitive decline in Alzheimer's disease. J Alzheimers Dis 2009;16:93–8.
[19] Shinde S, Pasupathy K. Respiratory-chain enzyme activities in isolated mitochondria of lymphocytes from patients with Parinson's diease: preliminary study. Neurol India 2006;54:390–3.
[20] Rana M, de Coo I, Diaz F, Smeets H, Moraes CT. An out-of-frame cytochrome b gene deletion from a patient with parkinsonism is associated with impaired complex III assembly and an increase in free radical production. Ann Neurol 2000;48:774–81.

PharmaNutrition 3 (2015) 136–145

Contents lists available at ScienceDirect

PharmaNutrition

journal homepage: www.elsevier.com/locate/phanu

Review

Clinical benefits of green tea consumption for cognitive dysfunction

Kazuki Ide, Hiroshi Yamada*

Department of Drug Evaluation & Informatics, Graduate School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan

ARTICLE INFO

Article history:
Available online 21 July 2015

Keywords:
Green tea
Catechin
Cognitive disorders
Epidemiology
Intervention studies

ABSTRACT

The prevalence of cognitive dysfunction, and particularly dementia, is increasing rapidly among older adults worldwide. There is currently no cure for dementia. In this situation, pharmaceutical and non-pharmaceutical combination therapies capable of preventing or slowing the progression of cognitive dysfunction are important. Nutritional intervention provides an important non-pharmaceutical approach in clinical practice. Green tea has the potential to contribute to this nutritional approach. Experimental studies *in vitro* and *in vivo* have suggested that green tea and its components could affect cognition *via* several potential mechanisms; these include its antioxidant and anti-inflammatory properties, protein kinase C activation, and acetylcholinesterase inhibition. Although several epidemiological and interventional studies in humans have suggested an association between tea consumption and cognition, not all studies have reported consistent findings. The present review summarizes experimental studies of the mechanisms involved in these effects and clinical studies of green tea consumption and cognition. This review provides a basis for the development of an evidence-based approach to the use of green tea and its ingredients in individuals with cognitive dysfunction.

Contents

Abbreviations: APP, amyloid precursor protein; EGCG, epigallocatechin-3-gallate; MCI, mild cognitive impairment; MMSE, mini-mental state examination; PKC, protein kinase C; ROS, reactive oxygen species.

* Corresponding author. Fax: +81 54 264 5762.
E-mail address: hyamada@u-shizuoka-ken.ac.jp (H. Yamada).

http://dx.doi.org/10.1016/j.phanu.2015.07.001
2213-4344/

1. Introduction

With increasing life expectancies worldwide, the prevalence of cognitive dysfunction, and dementia in particular, is increasing rapidly in older adults [1,2]. The age-standardized prevalence of dementia in individuals aged ≥60 years is 5–7% in most regions of the world, and the number of people with dementia has been predicted to almost double every 20 years [1]. Surprisingly, the number of individuals with dementia worldwide is estimated to reach 135.46 million in 2050 [2]. Although there are several pharmaceutical and non-pharmaceutical treatments for dementia, no fundamental curative therapy has been developed to date [3–9]. In this situation, pharmaceutical and non-pharmaceutical combination therapies aimed at slowing the progression of dementia are important. A consideration of risk factors is also important for the prevention of cognitive dysfunction, including dementia [10–12]. Epidemiological studies have suggested that nutritional and lifestyle factors can modify the risk of late-onset dementia [13–16]. Therefore, nutritional interventions have an important role in delaying the onset and slowing the progression of cognitive dysfunction.

Green tea is one of the foods with the potential to delay the onset of cognitive dysfunction or slow its progression [17–19]. Green tea is one of the most widely consumed beverages in the world, especially in Asian countries. It is produced from the fresh leaves of *Camellia sinensis*, and its chemical components include catechins and theanine, which have a variety of health benefits [20–22]. Their effects on cognitive function have been reported by experimental studies conducted *in vitro* and *in vivo*, and also from human epidemiological and interventional studies.

The present review briefly summarizes the pathophysiology of cognitive dysfunction associated with dementia and the psychoactive ingredients of green tea, and describes the possible mechanisms underlying the effects and the clinical benefits of green tea consumption on cognitive dysfunction.

2. Types of cognitive dysfunction and pathophysiology associated with dementia

2.1. Mild cognitive impairment

Mild cognitive impairment (MCI) is defined as a level of cognitive decline that is greater than expected for the individual's age and education level. Population-based epidemiological studies showed that 3–19% of adults older than 65 years of age had MCI [23]. MCI can represent a precursor to dementia, and more than 50% of individuals with MCI progress to dementia within 5 years [23–25]. This condition is classified into two subtypes; amnestic and non-amnestic MCI [26]. Amnestic MCI is more common than non-amnestic MCI [27], and Petersen et al. reported that more than 90% of individuals with amnestic MCI who progressed to dementia had signs of Alzheimer's disease [28]. The pathophysiology of amnestic MCI may involve a central cholinergic deficit and neural loss in the nucleus basalis of Meynert [29]. Amyloid-β, which is a trigger protein of Alzheimer's disease, is also involved in amnestic MCI; neurofibrillary tangles, mainly composed of hyperphosphorylated tau protein, are also increased [30]. Vascular risk factors are also involved in amnestic MCI [31]. Reduced cerebral perfusion leads to increased oxidative stress and neurodegeneration [32]. White matter abnormalities, asymptomatic infarcts, inflammation, and reduced glucose metabolism are also caused by vascular disease and increase the risk for cognitive impairment [33–35]. Non-amnestic MCI mainly affects attention, use of language, or visuospatial skills [36]. This condition is thought to represent a forerunner to frontotemporal lobar degeneration or dementia with Lewy bodies [26]; however, the pathophysiology of non-amnestic MCI is still unclear [37]. With regard to risk factors and pathological changes, vascular disease also increases the risk for non-amnestic MCI [36,38]. Differences in hippocampal atrophy between amnestic and non-amnestic MCI have been reported [39]. Green tea and its psychoactive ingredients may positively affect amnestic MCI through the potential mechanisms described in Sections 3.2–3.5, and non-amnestic MCI through the potential mechanisms described in Sections 3.2,3.4, and 3.5.

2.2. Alzheimer's disease

Alzheimer's disease is the most common cause of dementia, accounting for 60–80% of cases [40]. In this condition, neuronal loss occurs in specific brain regions, including the pyramidal cells in lamina II of the entorhinal cortex and in the CA1 region of the hippocampus; overall brain atrophy is also observed [8,41]. Neuronal loss and brain atrophy are caused by several pathways. Amyloid-β, derived from amyloid precursor protein (APP), is the trigger protein for Alzheimer's disease [42]. Autosomal dominant mutations in the APP, presenilin 1, and presenilin 2 genes affect APP processing to promote amyloid-β aggregation and accumulation in early onset Alzheimer's disease [43]. Oligomers of amyloid-β also have an important role in Alzheimer's disease pathology [44]. In addition, neurofibrillary tangles mainly composed of hyperphosphorylated tau protein, are associated with Alzheimer's disease [45]. Increased oxidative stress and neuroinflammation are also observed in this disease and may cause neurodegeneration and neuronal dysfunction [46–49]. Focusing on neurotransmission, the cholinergic system is of the utmost importance for modulating cognitive function in humans, and is a major target of medications for Alzheimer's disease, which temporarily improve symptoms [50]. In individuals with Alzheimer's disease, reduced acetylcholine esterase activity was observed in multiple cortical regions, and in the temporal and parietal cortices in particular [51]. Excitatory glutamatergic neurotransmission is also involved in learning, memory, and cognition. The *N*-methyl D-aspartate glutamate receptor is a target for Alzheimer's disease treatment [52], and reduced hippocampal glutamate levels have been reported in individuals with Alzheimer's disease [53]. Green tea and its psychoactive ingredients may positively affect Alzheimer's disease through the potential mechanisms described from Sections 3.2–3.5.

2.3. Vascular dementia

Vascular dementia is the second most common cause of dementia and is responsible for at least 20% of cases [54]. Some individuals also show mixed Alzheimer's disease and vascular dementia pathology [55]. A neuropathological association between cerebrovascular disease and vascular dementia has been reported [56,57], and individuals with large and multiple infarcts have an increased risk of developing dementia [58]; however, the underlying pathology is still elusive [54]. The brain requires a well-regulated blood supply to maintain all of its functions, and synaptic activity is particularly dependent on this [59,60]. Therefore, vascular structure and function are important in cognitive function. Other pathological studies have shown that oxidative stress and inflammation increase the risk of vascular cognitive impairment and/or dementia [61–63]. Considering these pathological aspects, green tea and its psychoactive ingredients may positively affect vascular dementia through the potential mechanisms described from Sections 3.2–3.5.

2.4. Dementia with Lewy bodies

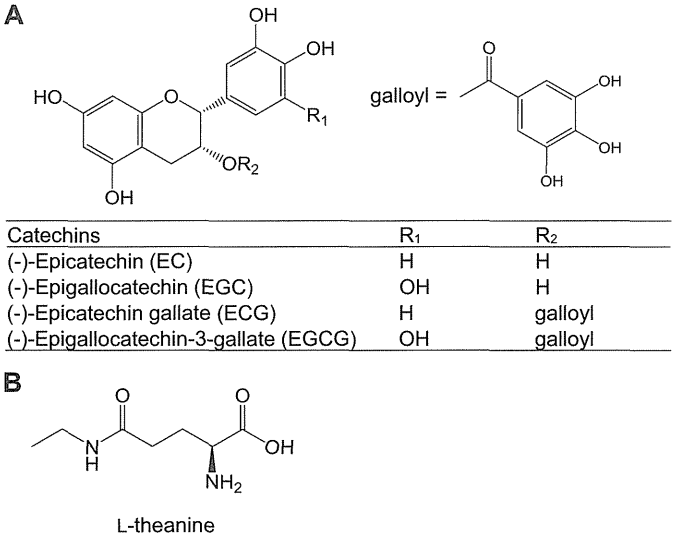
Dementia with Lewy bodies is also a common cause of dementia. Dementia with Lewy bodies has been identified in 10–15% of

dementia cases at autopsy [64], 4.2% of all dementia diagnosed in the community, and 7.5% of that diagnosed in a secondary care setting [65]. In dementia with Lewy bodies, severe impairment of visuospatial, attentional, and frontal-executive function is observed and memory impairment is less marked [66]. Indeed, an international diagnostic criterion for dementia with Lewy bodies requires cognitive impairment to precede or begin within a year of symptoms of parkinsonism [67]. Lewy bodies are the pathological hallmark of this cause of dementia. These develop through several phases from initial dust-like particles linked with α-synuclein, which aggregate to form dense ubiquitinated filaments and finally induce degeneration and death of the afflicted neurons [68]. Individuals with dementia with Lewy bodies also show neurochemical dysfunction, consistent with other types of dementia. Cholinergic and dopaminergic decline has been reported by a study using positron emission tomography [69]. Differences in the cholinergic deficit were reported to affect the symptoms of this condition [70]. Reduced levels of choline acetyltransferase were observed in the cortex [69–72], which appeared crucial for the development of cognitive dysfunction, in addition to the other symptoms described above. Considering these pathological aspects, green tea and its psychoactive ingredients might positively affect the cognitive impairment associated with dementia with Lewy bodies through the potential mechanisms described Sections 3.2, 3.4, and 3.5. However, the effects of green tea ingredients on Lewy body pathology have not been elucidated.

3. Experimental studies and possible mechanisms

3.1. Psychoactive ingredients of green tea

Green tea contains several psychoactive ingredients and some of these have potential health benefits in cognitive dysfunction. The main active ingredients of green tea are catechins and theanine [21] (Fig. 1). Catechins are present at particularly high levels in green tea, as compared with black tea or oolong tea, and epigallocatechin-3-gallate (EGCG) is a major green tea catechin. Lin et al. reported that Japanese green tea products contained 17.8 ± 0.90 mg (mean ± standard deviation) catechins (including 13.74 ± 0.82 mg EGCG)/100 mg dry leaves, while Chinese oolong tea (including paochong tea) contained 6.51 ± 0.57 mg catechins (including 4.97 ± 0.47 mg EGCG)/100 mg dry leaves [73]. They also showed that black tea contained less than one-fifteenth of the catechin level of longjing tea, which is categorized as a Chinese green tea, and approximately one-tenth of the level found in oolong tea [73]. Theanine is the only free amino acid in tea, and it is also present at high level in green tea [74]. Syu et al. have reported the theanine levels of different tea categories [74] and there is 15–20 mg theanine/g in dried tea leaves [75]. Caffeine is also a psychoactive ingredient of tea [76]. This is present at high levels in black teas, which contain approximately twice the levels found in green tea or oolong tea [73]. A large number of experimental studies on the effects of green tea and its ingredients have indicated the potential mechanisms underlying the health benefits of green tea consumption (Fig. 2). In this section, we have focused on the antioxidant/anti-inflammatory effects of green tea and its ingredients, as well as their influence on protein kinase C (PKC) activation and acetylcholine esterase inhibition. We have also briefly considered other possible mechanisms in Section 3.5.



Catechins	R_1	R_2
(-)-Epicatechin (EC)	H	H
(-)-Epigallocatechin (EGC)	OH	H
(-)-Epicatechin gallate (ECG)	H	galloyl
(-)-Epigallocatechin-3-gallate (EGCG)	OH	galloyl

Fig. 1. Chemical structures of green tea catechins and theanine. Catechins (A) and theanine (B).

3.2. Antioxidant and anti-inflammatory effects

Oxidative stress is considered to be involved in the etiology of several late-onset neurodegenerative disorders [77–79]. An imbalance between the levels of reactive oxygen species (ROS) and antioxidant agents produces oxidative stress [80]. The toxicity of ROS depends on the molecule involved, and there are several species including the superoxide and hydroxyl radicals and hydrogen peroxide [79,80]. These highly reactive molecules interact with biological molecules and the resultant damage can induce cellular dysfunction and degeneration [77–79]. One major pathway affected by oxidative stress is the proteasome-mediated cellular dysfunction and degeneration [81]. The proteasome is a multi-catalytic protease that plays a major role in ubiquitin-dependent and ubiquitin-independent protein turnover. Oxidative modification of the proteasome leads to impairment of this function, which impairs the efficient removal of potentially toxic proteins [82].

Biasibetti et al. reported that administration of 10 mg EGCG/kg/day for 4 weeks by oral gavage reversed oxidative stress induced by intracerebroventricular administration of streptozotocin in mice [83]. The results of this study suggested that EGCG had the potential to reverse a decrease in the activity of glutathione peroxidase, an enzyme that protects cells from oxidative stress and modulates expression of nitric oxide synthase. Nitric oxide is a signaling molecule that modulates vascular function and blood flow, and has also been shown to regulate neurotransmission in some settings [84]. It also forms reactive nitrogen species, and acts

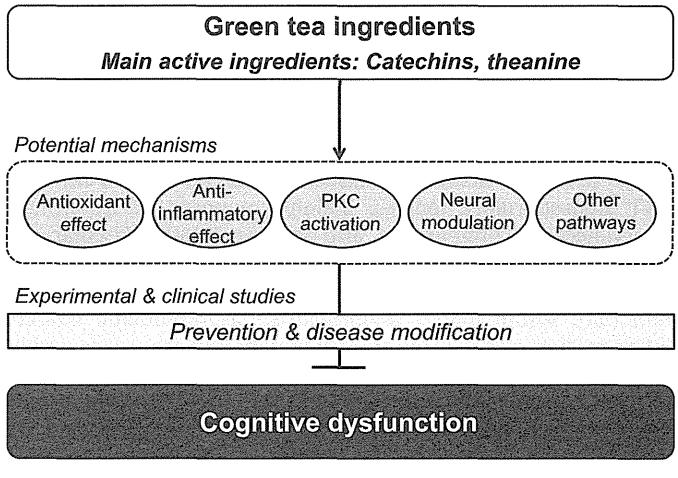


Fig. 2. Potential mechanisms underlying the effects of green tea and its ingredients on cognitive dysfunction.

as a pro- and anti-inflammatory molecule [85]. Unno et al. demonstrated that oral administration of 35 mg/kg/day of green tea catechin to senescence-accelerated mice (SAMP10) aged from 1 month to 15 months reduced their oxidative stress levels [86]. Performance in the passive avoidance task, which is widely used to evaluate contextual memory performance in rodents, was also improved by this treatment. In this context, a preventive effect of green tea catechins on the decline in glutathione peroxidase activity was reported in SAMP10 mice [87], and this may be related to the functional effects reported in the study. Reduced oxidative stress, with associated prevention of cognitive impairment by green tea and its components, was also reported by Wu et al. [88], Xu et al. [89], Haque et al. [90], Kim et al. [91], and Li et al. [92]. EGCG is known to activate two major enzymes that metabolize ROS, superoxide dismutase and catalase [93]; this may contribute to its antioxidant activities. The iron chelating activity of EGCG may also contribute to these effects [94,95]. Accumulation of iron in the brain with age not only affects neurodegeneration directly, but also contributes to oxidative stress [96–98]. Increased neuronal iron concentrations enhance susceptibility to toxins and activate pathogenic pathways, including morphological changes. Furthermore, iron contributes to the generation of ROS [98] and catalyzes oxidative reactions involved in the aggregation of α-synuclein [99], a major component of Lewy bodies.

Neuroinflammation plays an important role in the pathogenesis of several types of cognitive dysfunction, especially Alzheimer's disease [48,100]. Cross-talk between microglia and neurons may be involved in the pathophysiology of this condition. Cross-talk involving cytokines (including chemokines) could directly activate cellular cascades leading to neurodegeneration. For example, interleukin-1β, 6, and 12, and interferon γ and α, activate astrocytes and microglia [100]. Activated microglia proliferate and release cytotoxic factors, including ROS and reactive nitrogen species [101,102]. Therefore, microglial cells are thought to be a main source of pro-inflammatory factors, which contribute to neurodegeneration. Neuroinflammation has been reported to occur prior to neuronal cell loss [100] and intraperitoneal administration of lipopolysaccharide, a gram-negative bacterial cell wall component, caused neuroinflammation-induced memory impairment in mice. Lee et al. reported that oral administration of 1.5 and 3 mg/kg/day EGCG for 3 weeks prevented lipopolysaccharide-induced memory impairment [103]. Oral administration of EGCG also prevented astrocyte activation, elevation of cytokine levels, and the increase in inflammatory proteins; these were implicated in astrocyte activation and also in amyloidogenesis [103]. *In vitro* studies in BV-2 cells showed that EGCG inhibited cyclooxygenase-2 protein expression, which is induced by inflammatory stimuli [88]. Inhibition of nitric oxide production and inducible nitric oxide synthase protein expression by EGCG was also reported in BV-2 cells and primary microglia [88,104].

3.3. PKC pathway activation

PKC has a fundamental role in the regulation of cell survival and memory consolidation [105,106]. Enzymes involved in the PKC pathway have also shown neuroprotective effects against toxicity induced by amyloid-β peptides, which are involved in Alzheimer's disease. Levites et al. conducted an *in vitro* study using PC12 cells and reported that EGCG promoted non-toxic processing of APP by PKC-dependent activation of α-secretase [107]. In this study, a concentration-dependent effect was observed in the presence of 0.1–10 μM EGCG. An *in vivo* study of APP transgenic mice orally administered 2 mg EGCG/kg/day also supported this mechanism [107]. There are 12 isoforms of PKC [108], and EGCG has been shown to increase the levels of PKCα and ε in mouse hippocampus and striatum [107,109]. Overexpression of PKCε in APP transgenic mice decreased amyloid-β levels by increasing the expression of endothelin-converting enzyme, which degrades amyloid-β [110]. EGCG might therefore reduce amyloid-β toxicity by both stimulating non-toxic APP processing, and by promoting amyloid-β clearance. Recently, the importance of the PKCγ isoform activity in cognition has also been reported [111,112]; additional research regarding this, and the relationships between PKC isoforms, is needed.

3.4. Acetylcholinesterase inhibition

Deficits in cholinergic neurotransmission in the brain contribute to the cognitive decline observed in patients with dementia, and this is a major therapeutic target for Alzheimer's disease [8]. Cholinesterase inhibitors such as donepezil, rivastigmine, and galantamine are used to treat Alzheimer's disease in clinical practice [113]. Srividhya et al. demonstrated that the oral administration of 2 mg EGCG/kg/day for 30 days increased the level of acetylcholine in aged-rat brain cortex, and showed that EGCG formed a good docking-complex with acetylcholinesterase using *in silico* modeling [114]. The effects of EGCG as an acetylcholinesterase inhibitor were also reported by Biasibetti et al. in a streptozotocin mouse model [83]; Kim et al. in a scopolamine mouse model [115]; and Kaur et al. in young and old male Wistar rats [116]. Using *in vitro* approaches, Chung et al. reported that green tea polyphenols suppressed acetylcholinesterase activity in human neuroblastoma cells [117] and Okello et al. reported that a green tea extract inhibited human acetylcholinesterase activity, determined using a colorimetric assay [118].

Table 1
Characteristics of cross-sectional studies.

Source	Population	Types of tea	Outcomes	Reference no.
Kuriyama et al. 2006	1003 Japanese residents, aged ≥70 years	Green tea	1 (reference for ≤3 cups/week) *vs.* 0.46 (95% CI: 0.30, 0.72) for ≥2 cups/day, $P=0.0006$	[137]
Ng et al. 2008	2501 community-living Chinese residents, aged ≥55 years	Green tea, black tea, oolong tea	1 (reference for rare or no tea intake) *vs.* 0.56 (95% CI: 0.40, 0.78) for low level of intake; 1 *vs.* 0.45 (95% CI: 0.27, 0.72) for medium level of intake; 1 *vs.* 0.37 (95% CI: 0.14, 0.98) for high level of intake, $P<0.001$	[138]
Huang et al. 2009	681 Chinese residents, aged ≥90 years	Any type of tea	Lower prevalence of cognitive impairment in male but not female tea consumers, $P=0.041$ for former consumption, $P=0.044$ for current consumption	[139]
Nurk et al. 2009	2031 Norwegian residents, aged 70–74 years	Any type of tea (most common type during 1990s in Norway: black tea)	Dose-dependent effect of tea consumption on cognitive function (6 different cognitive tests) was observed up to ~200 ml/day, after which it plateaued or tended to be linear	[140]
Feng et al. 2010	716 Chinese residents, aged ≥55 years	Green tea, black tea, oolong tea	Total tea consumption was independently associated with better performances on global cognition, $P=0.03$; memory, $P=0.01$; executive function, $P=0.009$; and information processing speed, $P=0.001$	[141]

3.5. Other mechanisms

In addition to the properties described above, green tea and its components may exert neuroprotective effects through a variety of other mechanisms. These include green tea extract- and L-theanine-mediated regulation of the secretion of stress hormones that influence cognitive function, including corticosterone [119,120]; EGCG-mediated reduction of amyloid-induced mitochondrial dysfunction [121]; inhibition of glutamate dehydrogenase [122,123], 3,4-dihydroxyphenylalanine decarboxylase [124], and histone acetyltransferase [125]; inhibition of fatty acid synthase by catechins [126,127]; interactions between catechin derivatives and opioid/cannabinoid receptors [128,129]; and L-theanine-mediated modulation of glutamatergic [130], GABAergic [131], dopaminergic [132], and serotoninergic neurotransmission in the brain [132–134]. Caffeine is also a psychoactive ingredient of green tea [76] and this stimulates cholinergic neurons by acting as a non-selective antagonist of adenosine A1 and A2A receptors [135]. Adenosine A2A receptor expression is also regulated by caffeine consumption [136], and these effects may alter cognitive function.

4. Epidemiological studies

4.1. Cross-sectional studies

Five cross-sectional studies have been reported; these were conducted in Japan, China, and Norway [137–141] and their study populations and findings are summarized in Table 1.

The Kuriyama et al. study was conducted in north-east Japan (Tohoku region) and included 1003 Japanese residents [137]. Cognitive function was evaluated by the mini-mental state examination (MMSE), a well-validated test of cognitive function that is widely used in clinical practice [142]. This study identified an association between a higher consumption of green tea and a lower prevalence of cognitive impairment in Japanese participants aged ≥70. Compared with the participants consuming ≤3 cups green tea/week (reference), the adjusted odds ratios for cognitive impairment (MMSE score <26/30) were 0.62 (95% confidence interval [CI]: 0.33, 1.19) for 4–6 cups/week or 1 cup/day, and 0.46 (95% CI 0.30, 0.72) for ≥2 cups/day. The study also showed no association between MMSE scores and the consumption of oolong, black tea, or coffee.

The study by Nurk et al. used data from the Hordaland Health Study, conducted in western Norway [140]. A total of 2031 participants aged from 70 to 74 were included in this study, which focused on the relationship between consumption of flavonoid-rich food (tea, chocolate, and wine) and cognitive function (evaluated by MMSE). There was an association between flavonoid-rich food consumption and higher cognitive performance. The kind of tea consumed was not surveyed in this study; however, the authors wrote that black tea was commonly consumed in Norway in the 1990s. Associations between tea consumption and cognitive function were also reported in other studies [138,139,141]. However, one study by Huang et al. identified a sex-related difference in this effect [139], whereby tea consumption was only associated with cognition in men included in this study of Chinese nonagenarians/centenarians.

4.2. Longitudinal studies

Data have been reported from 5 longitudinal studies conducted in Japan, China, Finland, and the U.S. [138,141,143–145]. These studies are summarized in Table 2.

The study reported by Noguchi-Shinohara et al. was conducted in central Japan (Hokuriku region) and included 723 Japanese residents aged >60 years [145]. The study was conducted from 2007, with 4.9 ± 0.9 years follow-up (mean ± standard deviation). Cognitive function was assessed by MMSE and the clinical dementia rating, which uses a semi-structured interview with the patient and an informant to rate cognitive impairment [146]. This study reported that the group who drank green tea habitually had a lower incidence of dementia or MCI, as compared with the control group who did not drink green tea. No association was observed between the consumption of black tea or coffee and the incidence of dementia or MCI.

The Ng et al. study was conducted in China with 1438 Chinese residents aged ≥55 who participated in the Singapore longitudinal aging studies [138]. It was conducted from 2003, with 1–2 years follow-up (median: 16 months). Cognitive impairment was defined as an MMSE score ≤23, and cognitive decline was defined as a decrease in MMSE score of ≥1 point. This study identified an association between higher tea consumption and lower prevalence of cognitive impairment, even when adjusted for confounding variables such as age, sex, education, lifestyle factors, complications, and other risk factors. The Feng et al. study also showed a similar association [147]. However, a study conducted in Finland and reported by Eskelinen et al. showed no association between mid-life tea consumption and late-life prevalence of dementia, including Alzheimer's disease [143]. Another study reported by Arab et al. showed a sex-related difference [144], whereby tea consumption modestly reduced the rate of cognitive decline in women, but not in men. Taken together, these studies indicated that the association between higher green tea consumption and a lower prevalence of cognitive dysfunction is still controversial.

Table 2
Longitudinal studies.

Source	Population description	Types of tea	Outcomes	Reference no.
Ng et al. 2008	1438 Chinese residents, aged ≥55, 1- to 2-year follow-up	Green tea, black tea, oolong tea	Less decline of cognitive function in participants with higher level of tea consumption, $P = 0.042$	[138]
Eskelinen et al. 2009	1409 Finnish residents, aged 65–79, 21-year follow-up	Any type of tea	No association with dementia or Alzheimer's disease	[143]
Arab et al. 2011	4809 U.S. residents, aged ≥65, 7.9-year follow-up	Any type of tea	Reduced rate of cognitive decline in women, $P = 0.07$ for modified MMSE, $P = 0.04$ for modified MMSE with item response theory; non-linear relationship with the frequency of tea consumption	[144]
Feng et al. 2012	7139 Chinese residents, aged 80–115, 7-year follow-up	Any type of tea	Higher verbal frequency scores throughout the follow-up period; but steeper slope of cognitive decline compared with non-drinker from a higher baseline level; coefficient for the interaction term Time × Daily drinking = −0.12, $P = 0.02$	[147]
Noguchi-Shinohara et al. 2014	723 Japanese residents, aged≥ 60 years, 4.9-year follow-up	Green tea	Incidence of overall cognitive decline (dementia or mild cognitive impairment): 0.32 (95% CI: 0.16–0.64) for everyday drinker; 0.47 (95% CI: 0.25–0.86) for 1–6 days/week drinker *vs.* 1 (reference, non-drinker)	[145]

Table 3
Interventional studies.

Source	Population description	Intervention	Outcomes	Reference no.
Kataoka et al. 2009	29 Japanese participants with cognitive dysfunction, aged 85 years on average	Green tea with high theanine content (2040 mg/day) *vs.* placebo, 12 months	Improvement of cognitive function based on revised Hasegawa dementia scale, $P < 0.05$	[148]
Park et al. 2011	91 Chinese participants with mild cognitive impairment, aged 40–75 years	Green tea-based dietary supplement (LGNC-07, 1680 mg/day) *vs.* placebo, 4 months	Improvement of cognitive function based on Rey–Kim memory test (memory) in 16 weeks, $P = 0.0478$; and Stroop test (attention) in 8 weeks, $P = 0.0306$	[149]
Ide et al. 2014	12 Japanese participants with dementia, aged ≥65 years	Green tea powder (2000 mg/day), 3 months	Improvement of cognitive function based on MMSE Japanese version *vs.* before intervention, $P = 0.03$	[150]

5. Interventional studies

There have been 3 reports of interventional studies conducted in Japan and Korea [148–150]. The characteristics of these studies are summarized in Table 3.

The Park et al. study published in 2011 was conducted in Korea and included 91 Korean participants with MCI [149], defined as a score between 21 and 26 on the Korean version of the MMSE. The participants took 1680 mg/day of a dietary supplement containing green tea extract and L-theanine (LGNC-07) for 16 weeks. This corresponded to a daily dose of 1440 mg green tea extract and 240 mg L-theanine. Neuropsychological tests (Rey–Kim memory test [151] and Stroop color-word test [152]) were used to evaluate memory and attention. After consuming LGNC-07 for 16 weeks, Rey–Kim memory test scores were significantly improved in participants with a Korean MMSE score of 21–23. This difference was only observed in an analysis that was stratified by the baseline MMSE score, and was not observed in all participants. Kataoka et al. also reported a preventive effect of green tea with a high theanine content on declining performance in the revised Hasegawa dementia scale score [148]. These studies employed green tea-like dietary supplements, but it is also important to study the effects of daily green tea consumption patterns on cognition.

We conducted a study in Japan in 2012 using normal green tea powder [150]. This study included 12 participants with cognitive dysfunction, defined as a score of <28 on the Japanese version of the MMSE. The participants consumed 2000 mg green tea powder/day, containing 227 mg catechins and 42 mg theanine, for 3 months. After this period, individual MMSE scores were significantly improved (before, 15.3 ± 7.7; after, 17.0 ± 8.2; $P = 0.03$).

All of these interventional studies suggested that consumption of green tea or green tea-based dietary supplements may reduce the rate of cognitive decline or even improve cognitive function in people with established cognitive impairments. However, only a limited number of small-scale studies have been conducted, and additional large-scale randomized controlled studies are needed to determine the effects of green tea consumption on cognition.

6. Conclusions and future perspectives

Green tea and its components have the potential to modify cognitive disorders and a variety of potential mechanisms have been identified using *in vitro* and *in vivo* approaches. These approaches used cell lines and rodent models, and the key mechanisms involved in humans remain unclear. Several epidemiological and interventional studies in humans demonstrated that consumption of green tea or its components may delay the onset and/or reduce the progression of dementia and MCI. Among epidemiological studies, 3 cross-sectional studies and 2 longitudinal studies analyzed the types of tea consumed. However, the results of these studies were not always consistent. The ingredients of tea can differ, depending on the manufacturing process and on fermentation in particular; these differences may affect the associations observed between tea consumption and cognitive function. A limited number of interventional studies have been reported and these were small-scale; further large-scale randomized controlled studies are needed to elucidate the effects of green tea consumption on cognitive function. Previous studies did not consider the pharmacodynamic properties of the active ingredients of green tea. Consideration of these in future studies should help to clarify the effects of green tea in humans and the mechanisms underlying these activities.

A recent functional magnetic resonance imaging study by Schmidt et al. revealed that green tea extracts enhanced parieto-frontal connectivity in healthy volunteers [153]. Parieto-frontal connectivity contributes to working memory processing [154] and may therefore be involved in the effects of green tea on cognitive dysfunction. Additional human studies of the functional and pathophysiological changes associated with cognitive dysfunction are expected to improve understanding of these processes.

Zhang et al. reported that green tea catechins increased the effects of the acetylcholinesterase inhibitor, huperzine A, by increasing its affinity for serum albumin [155]. This study highlighted the possibility that green tea catechins may act to enhance the effects of other medications. In addition to the studies focusing on the effects of the active ingredients of green tea, studies of pharma-nutritional interactions in humans will also facilitate effective nutritional interventions using green tea and its ingredients in clinical practice.

Author contributions

Kazuki Ide and Hiroshi Yamada searched the literature and drafted the manuscript.

Acknowledgements

This work was supported in part by a grant from the Japanese Ministry of Health, Labor and Welfare (No. 242 20501 to HY) and a grant from the Japan Society for the Promotion of Science (JSPS), through the Grant-in-Aid for JSPS Fellows (No. 15J10190 to KI).

References

[1] Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement. 2013;9(1):63–75, doi:http://dx.doi.org/10.1016/j.jalz.2012.11.007. 23305823.

[2] Prince M, Guerchet M, Prina M. Alzheimer's disease international. Policy brief for heads of government: the global impact of dementia 2013–2050. Alzheimer's Dis. Int. 2013.

[3] EFNS Scientist Panel on Dementia and Cognitive Neurology. Sorbi S, Hort J, Erkinjuntti T, Fladby T, Gainotti G, Gurvit H, Nacmias B, Pasquier F, Popescu BO, Rektorova I, Religa D, Rusina R, Rossor M, Schmidt R, Stefanova E, Warren JD, Scheltens P. EFNS-ENS guidelines on the diagnosis and management of disorders associated with dementia. Eur. J. Neurol. 2012;19(9):1159–79, doi:http://dx.doi.org/10.1111/j.1468-1331.2012.03784.x. 22891773.

[4] Sadowsky CH, Galvin JE. Guidelines for the management of cognitive and behavioral problems in dementia. J. Am. Board Fam. Med. 2012;25(3):350–66, doi:http://dx.doi.org/10.3122/jabfm.2012.03.100183. 22570399.
[5] Ballard C, Khan Z, Clack H, Corbett A. Nonpharmacological treatment of Alzheimer disease. Can. J. Psychiatry 2011;56(10):589–95. 22014691.
[6] Teixeira CV, Gobbi LT, Corazza DI, Stella F, Costa JL, Gobbi S. Non-pharmacological interventions on cognitive functions in older people with mild cognitive impairment (MCI). Arch. Gerontol. Geriatr. 2012;54(1):175–80, doi:http://dx.doi.org/10.1016/j.archger.2011.02.014. 21397960.
[7] Cotelli M, Manenti R, Zanetti O. Reminiscence therapy in dementia: a review. Maturitas 2012;72(3):203–5, doi:http://dx.doi.org/10.1016/j.maturitas.2012.04.008. 22607813.
[8] Huang Y, Mucke L. Alzheimer mechanisms and therapeutic strategies. Cell 2012;148(6):1204–22, doi:http://dx.doi.org/10.1016/j.cell.2012.02.040. 22424230.
[9] Salomone S, Caraci F, Leggio GM, Fedotova J, Drago F. New pharmacological strategies for treatment of Alzheimer's disease: focus on disease modifying drugs. Br. J. Clin. Pharmacol. 2012;73(4):504–17, doi:http://dx.doi.org/10.1111/j.1365-2125.2011.04134.x. 22035455.
[10] Etgen T, Sander D, Bickel H, Förstl H. Mild cognitive impairment and dementia: the importance of modifiable risk factors. Dtsch. Arztebl. Int. 2011;108(44):743–50, doi:http://dx.doi.org/10.3238/arztebl.2011.0743. 22163250.
[11] Reitz C, Mayeux R. Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. Biochem. Pharmacol. 2014;88(4):640–51, doi:http://dx.doi.org/10.1016/j.bcp.2013.12.024. 24398425.
[12] Daviglus ML, Plassman BL, Pirzada A, Bell CC, Bowen PE, Burke JR, Connolly Jr. ES, Dunbar-Jacob JM, Granieri EC, McGarry K, Patel D, Trevisan M, Williams Jr. JW. Risk Factors and Preventive Interventions for Alzheimer Disease State of the Science. Arch Neurol 2011;68(9):1185–90, doi:http://dx.doi.org/10.1001/archneurol.2011.100. 21555601.
[13] Ozawa M, Ninomiya T, Ohara T, Doi Y, Uchida K, Shirota T, Yonemoto K, Kitazono T, Kiyohara Y. Dietary patterns and risk of dementia in an elderly Japanese population: the Hisayama study. Am. J. Clin. Nutr. 2013;97(5):1076–82, doi:http://dx.doi.org/10.3945/ajcn.112.045575. 23553168.
[14] Xu WL, Atti AR, Gatz M, Pedersen NL, Johansson B, Fratiglioni L. Midlife overweight and obesity increase late-life dementia risk: a population-based twin study. Neurology 2011;76(18):1568–74, doi:http://dx.doi.org/10.1212/WNL.0b013e3182190d09. 21536637.
[15] Simons LA, Simons J, McCallum J, Friedlander Y. Lifestyle factors and risk of dementia: Dubbo study of the elderly. Med. J. Aust. 2006;184(2):68–70. 16411871.
[16] von Arnim CAF, Gola U, Biesalski HK. More than the sum of its parts? Nutrition in Alzheimer's disease. Nutrition 2010;26(7–8):694–700, doi:http://dx.doi.org/10.1016/j.nut.2009.11.009. 20381316.
[17] Mandel SA, Avramovich-Tirosh Y, Reznichenko L, Zheng H, Weinreb O, Amit T, Youdim MB. Multifunctional activities of green tea catechins in neuroprotection. Modulation of cell survival genes, iron-dependent oxidative stress and PKC signaling pathway. Neurosignals 2005;14(1–2):46–60, doi:http://dx.doi.org/10.1159/000085385. 15956814.
[18] Mandel SA, Amit T, Weinreb O, Reznichenko L, Youdim MB. Simultaneous manipulation of multiple brain targets by green tea catechins: a potential neuroprotective strategy for Alzheimer and Parkinson diseases. CNS Neurosci. Ther. 2008;14(4):352–65, doi:http://dx.doi.org/10.1111/j.1755-5949.2008.00060.x. 19040558.
[19] Song J, Xu H, Liu F, Feng L. Tea and cognitive health in late life: current evidence and future directions. J. Nutr. Health Aging 2012;16(1):31–4, doi:http://dx.doi.org/10.1007/s12603-011-0139-9. 22237999.
[20] Cabrera C, Artacho R, Giménez R. Beneficial effects of green tea—a review. J. Am. Coll. Nutr. 2006;25(2):79–99, doi:http://dx.doi.org/10.1080/07315724.2006.10719518. 16582024.
[21] Chacko SM, Thambi PT, Kuttan R, Nishigaki I. Beneficial effects of green tea: a literature review. Chin. Med. 2010;5:13, doi:http://dx.doi.org/10.1186/1749-8546-5-13. 20370896.
[22] Johnson R, Bryant S, Huntley AL. Green tea and green tea catechin extracts: an overview of the clinical evidence. Maturitas 2012;73(4):280–7, doi:http://dx.doi.org/10.1016/j.maturitas.2012.08.008. 22986087.
[23] International Psychogeriatric Association Expert Conference on mild cognitive impairment. Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, Belleville S, Brodaty H, Bennett D, Chertkow H, Cummings JL, de Leon M, Feldman H, Ganguli M, Hampel H, Scheltens P, Tierney MC, Whitehouse P, Winblad B. Mild cognitive impairment. Lancet 2006;367(9518):1262–70, doi:http://dx.doi.org/10.1016/S0140-6736(06)68542-5. 16631882.
[24] Bruscoli M, Lovestone S. Is MCI really just early dementia? A systematic review of conversion studies. Int. Psychogeriatr. 2004;16(2):129–40, doi:http://dx.doi.org/10.1017/S1041610204000092. 15318760.
[25] Medical Research Council. Matthews FE, Stephan BC, McKeith IG, Bond J, Brayne C. Two-year progression from mild cognitive impairment to dementia: to what extent do different definitions agree? J. Am. Geriatr. Soc. 2008;56(8):1424–33, doi:http://dx.doi.org/10.1111/j.1532-5415.2008.01820.x. 18662209.
[26] Petersen RC. Mild cognitive impairment as a diagnostic entity. J. Intern. Med. 2004;256(3):183–94, doi:http://dx.doi.org/10.1111/j.1365-2796.2004.01388.x. 15324362.
[27] Petersen RC, Roberts RO, Knopman DS, Geda YE, Cha RH, Pankratz VS, Boeve BF, Tangalos EG, Ivnik RJ, Rocca WA. Prevalence of mild cognitive impairment is higher in men. The Mayo Clinic Study of Aging. Neurology 2010;75(10):889–97, doi:http://dx.doi.org/10.1212/WNL.0b013e3181f11d85. 20820000.
[28] Alzheimer's Disease Cooperative Study Group. Petersen RC, Thomas RG, Grundman M, Bennett D, Doody R, Ferris S, Galasko D, Jin S, Kaye J, Levey A, Pfeiffer E, Sano M, van Dyck CH, Thal LJ. Vitamin E and donepezil for the treatment of mild cognitive impairment. N. Engl. J. Med. 2005;352(23):2379–88, doi:http://dx.doi.org/10.1056/NEJMoa050151. 15829527.
[29] Mufson EJ, Ma SY, Cochran EJ, Bennett DA, Beckett LA, Jaffar S, Saragovi HU, Kordower JH. Loss of nucleus basalis neurons containing trkA immunoreactivity in individuals with mild cognitive impairment and early Alzheimer's disease. J. Comp. Neurol. 2000;427(1):19–30, doi:http://dx.doi.org/10.1002/1096-9861(20001106)427:1<19::AID-CNE2>3.0.CO;2-A. 11042589.
[30] Mufson EJ, Binder L, Counts SE, DeKosky ST, de Toledo-Morrell L, Ginsberg SD, Ikonomovic MD, Perez SE, Scheff SW. Mild cognitive impairment: pathology and mechanisms. Acta Neuropathol. 2012;123(1):13–30, doi:http://dx.doi.org/10.1007/s00401-011-0884-1. 22101321.
[31] Bennett DA, Schneider JA, Bienias JL, Evans DA, Wilson RS. Mild cognitive impairment is related to Alzheimer disease pathology and cerebral infarctions. Neurology 2005;64(5):834–41, doi:http://dx.doi.org/10.1212/01.WNL.0000152982.47274.9E. 15753419.
[32] de la Torre JC. Alzheimer disease as a vascular disorder—nosological evidence. Stroke 2002;33(4):1152–62, doi:http://dx.doi.org/10.1161/01.STR.0000014421.15948.67. 11935076.
[33] Jani B, Rajkumar C. Ageing and vascular ageing. Postgrad. Med. J. 2006;82(968):357–62, doi:http://dx.doi.org/10.1136/pgmj.2005.036053. 16754702.
[34] Nilsson PM. Early vascular aging (EVA): consequences and prevention. Vasc. Health Risk Manag. 2008;4(3):547–52. 18827905.
[35] O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, Bowler JV, Ballard C, DeCarli C, Gorelick PB, Rockwood K, Burns A, Gauthier S, DeKosky ST. Vascular cognitive impairment. Lancet Neurol. 2003;2(2):89–98, doi:http://dx.doi.org/10.1016/S1474-4422(03)00305-3. 12849265.
[36] Molano J, Boeve B, Ferman T, Smith G, Parisi J, Dickson D, Knopman D, Graff-Radford N, Geda Y, Lucas J, Kantarci K, Shiung M, Jack C, Silber M, Pankratz VS, Petersen R. Mild cognitive impairment associated with limbic and neocortical Lewy body disease: a clinicopathological study. Brain 2010;133(2):540–56, doi:http://dx.doi.org/10.1093/brain/awp280. 19889717.
[37] Reinvang I, Grambaite R, Espeseth T. Executive dysfunction in MCI: subtype or early symptom. Int. J. Alzheimers Dis. 2012;2012:936272, doi:http://dx.doi.org/10.1155/2012/936272. 22693679.
[38] Jungwirth S, Zehetmayer S, Hinterberger M, Tragl KH, Fischer P. The validity of amnestic MCI and non-amnestic MCI at age 75 in the prediction of Alzheimer's dementia and vascular dementia. Int. Psychogeriatr. 2012;24(6):959–66, doi:http://dx.doi.org/10.1017/S1041610211002870. 22300486.
[39] Kantarci K, Petersen RC, Przybelski SA, Weigand SD, Shiung MM, Whitwell JL, Negash S, Ivnik RJ, Boeve BF, Knopman DS, Smith GE, Jack Jr. CR. Hippocampal volumes, proton magnetic resonance spectroscopy metabolites, and cerebrovascular disease in mild cognitive impairment subtypes. Arch. Neurol. 2008;65(12):1621–8, doi:http://dx.doi.org/10.1001/archneur.65.12.1621.19064749.
[40] Alzheimer's Association. Alzheimer's disease facts and figures. Alzheimers Dement. 2014;2014(10):e47–92.
[41] Gómez-Isla T, Price JL, McKeel DW, Morris JC, Growdon JH, Hyman BT. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. J. Neurosci. 1996;16(14):4491–500. 8699259.
[42] Palop JJ, Mucke L. Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat. Neurosci. 2010;13(7):812–8, doi:http://dx.doi.org/10.1038/nn.2583. 20581818.
[43] Bertram L, Lill CM, Tanzi RE. The genetics of Alzheimer disease: back to the future. Neuron 2010;68(2):270–81, doi:http://dx.doi.org/10.1016/j.neuron.2010.10.013. 20955934.
[44] Lesné SE, Sherman MA, Grant M, Kuskowski M, Schneider JA, Bennett DA, Ashe KH. Brain amyloid-beta oligomers in ageing and Alzheimer's disease. Brain 2013;136(5):1383–98, doi:http://dx.doi.org/10.1093/brain/awt062.23576130.
[45] Rudrabhatla P, Jaffe H, Pant HC. Direct evidence of phosphorylated neuronal intermediate filament proteins in neurofibrillary tangles (NFTs): phosphoproteomics of Alzheimer's NFTs. FASEB J. 2011;25(11):3896–905, doi:http://dx.doi.org/10.1096/fj.11-181297. 21828286.
[46] Zhao Y, Zhao B. Oxidative stress and the pathogenesis of Alzheimer's disease. Oxid. Med. Cell. Longev. 2013;2013:316523, doi:http://dx.doi.org/10.1155/2013/316523. 23983897.
[47] Zotova E, Nicoll JA, Kalaria R, Holmes C, Boche D. Inflammation in Alzheimer's disease: relevance to pathogenesis and therapy. Alzheimers Res. Ther. 2010;2(1):1, doi:http://dx.doi.org/10.1186/alzrt24. 20122289.
[48] Pasqualetti G, Brooks DJ, Edison P. The role of neuroinflammation in dementias. Curr. Neurol. Neurosci. Rep. 2015;15(4):17, doi:http://dx.doi.org/10.1007/s11910-015-0531-7. 25716012.
[49] Schrag M, Mueller C, Zabel M, Crofton A, Kirsch WM, Ghribi O, Squitti R, Perry G. Oxidative stress in blood in Alzheimer's disease and mild cognitive impairment: a meta-analysis. Neurobiol. Dis. 2013;59:100–10, doi:http://dx.doi.org/10.1016/j.nbd.2013.07.005. 23867235.