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Original Research

Protective properties of complex of quercetin, selenium, catechins and curcumin against DNA damage

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Abstract

Nutrition plays an important role in prevention of degenerative age-related diseases. Sufficient intake of bioactive compounds such as polyphenols, vitamins, minerals, fiber, unsaturated fat with antisclerotic, antioxidative, antiinflammatory and anticarcinogenic properties in form of functional foods or separately is a correct way of this prevention. In a group of randomly selected 50 apparently healthy non-smoking men aged 30-50 years on traditional mixed diet (general population) plasma concentrations of antioxidant vitamins, catechins, selenium and lymphocyte DNA damage values were measured before consumption of cereal biscuit with selenized onion, green tea and curcuma as the sources of bioactive quercetin, selenium, catechins, curcumin, after 2-month consumption and after 2-month wash-out. The significantly increased plasma concentrations of biscuit antioxidants (selenium, catechins) after 2-month consumption and the non-changed antioxidant vitamins and carotenoids (vitamins C, E, β-carotene and lycopene) during the study mean that the biscuit antioxidants (selenium, catechins and non-measured quercetin, curcumin but present in biscuit) actually caused the significantly reduced values of DNA strand breaks, DNA breaks with oxidized purines and DNA breaks with oxidized pyrimidines. After two months wash-out period, a trend to return of these values derived from biscuit as well as DNA damage values to levels before consumption was expressed. The results of reduced values of DNA damage document a beneficial effect of biscuit as functional food containing polyphenols with antioxidant and anticarcinogenic properties in prevention of DNA instability and prevention of oxidative stress.

INTRODUCTION

Nutrition is a key environmental factor influencing the incidence of many chronic diseases mainly the widely occurred cardiovascular disease and cancer. The observed growth of cancer incidence has been usually linked to frequent consumption of "unhealthy" food [1]. Such food often contains genotoxic substances as heterocyclic aromatic amines or polycyclic aromatic hydrocarbons, occurring during food preparation, which induce an accumulation of unrepaired damage, initiating DNA instability and cancer development [1]. A diet containing plenty of fruits and vegetables, due to the presence of biologically active polyphenols, can modulate activity of detoxifying enzymes [1-3]. Such a diet can decrease the extent DNA adducts, breaks and oxidative damage, supporting the body enzymatic system in sufficient removal of DNA damage. The antioxidant vitamins and other antioxidant compounds in consumed foods also enhance the DNA protection by the scavenging of radical oxidative species that occur in incorrectly culinary prepared food or during metabolic reactions. The endogenous antioxidants are inadequate to prevent damage completely, so that diet-derived antioxidants have an important role in maintaining health [2].

The acceptable recommendations for nutritional prevention of degenerative age-related diseases are sufficient and regular intakes of bioactive compounds (in form of functional foods or separately) with expressive antisclerotic, antioxidative and antiinflammatory properties. Functional foods are defined such as food or food compounds which have the health beneficial preventive effects related to degenerative age-related diseases [4]. Plants are rich in bioactive compounds such as polyphenols (mainly flavonoids such as flavonols, quercetin, catechins and phytoestrogens), vitamins, mineral compounds, fibers with favourable effects on genetic, cellular, biochemical and physiological functions of the organism [4].

The functional food (cereal biscuit with bioactive complex of selenium in organic form, quercetin, curcumin, catechins presented in natural plant sources) selenized onion, curcuma and green tea was produced and consumed by volunteers for health benefits evaluation of these compounds together in one complex. The aim of this study was to assess the changes in DNA damage products after two month consumption of introduced biscuit.

SUBJECTS AND METHODS

Randomly selected group of apparently healthy non-smoking men aged 30-50 years on traditional mixed diet (general population) was used in intervention study of evaluation of health effects of cereal biscuit consumption. The characteristic of the study group is shown in Table 1. The volunteers (workers of Slovak Medical University and subjects from previous university projects) consumed 100 g of biscuit daily during 2 month. They were informed that their usual nutritional habits during biscuit consumption have not to be changed. Before consumption of biscuit the volunteers recorded into standardized and validated questionnaires their nutritional habit during three days. The basic somatometric, biochemical and hematological parameters were measured before consumption (Table 1). The Regional Ethic Committee approved the study, and all participants gave their written informed consent.

Onion was selenium fortified by watering of onion fields with selenized water in the form of sodium selenate. Dried onion together with green tea and curcuma as the main natural sources of bioactive compounds were used on the production of biscuit, which was realized by common technology in Bakery factory, Liptovsky Hradok, Slovakia. The selection of amounts of bioactive compounds and other food items of biscuit was realized according to literature data as well as in cooperation with research workers of the factory having the knowledge and experience with technology of biscuit productions. The composition of biscuit is given in Table 2.

Blood was sampled after an overnight fasting using a standard procedure. EDTA was used as an anticoagulant. Concentrations of vitamins C, E and carotenoids in plasma (beta-carotene, lycopene) were detected by high-performance liquid chromatographic method (HPLC) [5, 6]. Graphite furnace atomic absorption spectrometry (GFAAS) was used for direct selenium determination in serum [7]. Selenium concentration in biscuit was measured after microwave oven biscuit decomposition [7]. Concentrations of catechins in plasma (epicatechin, epigallocatechin gallate, epicatechin gallate) were detected by HPLC [8].

Alkaline comet assay was used for detection of DNA strand breaks, oxidized purines and oxidized pyrimidines in isolated lymphocytes. For detection of oxidized purines, slides were incubated with formamidopyrimidine glycosylase, oxidized pyrimidines were detected after incubation with endonuclease III. Comets were analyzed by visual scoring of 100 randomly selected images per gel, classifying them into five categories representing relative tail intensity and thus increasing degrees of damage. This method was calibrated by reference to computer image analysis based on fluorometric measurement of DNA intensities in head and tail [9].

Table 1. Characteristic of group of volunteers

Parameters	Mean ± SEM
n	50
Age range (years)	30-50
Body mass index (kg/m^2)	26.5 ± 0.5
WHR	0.887 ± 0.008
Blood pressure (mmHg)	138 ± 2 / 83 ± 1
% of body fat	23.1 ± 0.8
Total cholesterol (mmol/l)	5.17 ± 0.14
LDL-cholesterol (mmol/l)	3.18 ± 0.12
HDL-cholesterol (mmol/l)	1.25 ± 0.04
Triacylglycerols (mmol/l)	1.68 ± 0.15
Glucose (mmol/l)	5.01 ± 0.06
Albumin (g/l)	48.1 ± 0.3
Calcium (mmol/l)	2.51 ± 00.01
Magnesium (mmol/l)	0.76 ± 0.01
Potassium (mmol/l)	4.63 ± 0.05
Iron (μmol/l)	19.5 ± 1.03
Amylase (μkat/l)	0.91 ± 0.03
AST (μkat/l)	0.59 ± 0.04
ALT (μkat/l)	0.51 ± 0.03
ALP (μkat/l)	1.4 ± 0.07
Total bilirubin (μmol/l)	13.1 ± 0.6
Leukocytes (x10^9/l)	6.9 ± 0.22
Erythrocytes (x10^{12}/l)	5.2 ± 0.18
Platelets (x10^9/l)	220 ± 7
Hemoglobin (g/l)	156±2

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Table 2. Composition of 100 g of cereal biscuit

Nutritional compounds and nutrients	Amount
Dried selenized onion	6 g
Green tea	2 g
Curcuma	1.3 g
Plant fat	19 g
Whole grain oat flour	35 g
Oat bran	10 g
Linen seed	3 g
+++	
Proteins	11.2 g
Saccharides	52.4 g
Fiber	19.7 g
Lipids	24.4 g
Saturated fatty acids	14.6 g
Monounsaturated fatty acids	7.1 g
Oleic acid	6.73 g
Polyunsaturated fatty acids	2.7 g
Linoleic acid	2.46 g
alpha-linolenic acid	0.19 g
Trans-fatty acids	0.22 g
Selenium	115 µg

+++ Other biscuit compounds: whey, dried eggs, guar gum, salt, cumin, natrium hydrocarbonate, amonium hydrocarbonate.

Serum concentrations of total cholesterol, HDL-cholesterol, triacylglycerols, glucose, albumin, iron, magnesium, potassium, calcium, total bilirubin and liver enzyme activities were measured by standard laboratory methods on Vitros 250 autoanalyzer (Johnson & Johnson, New York, NY, USA). Value of LDL-cholesterol was calculated according to Friedewald formula:

LDL-cholesterol =
Total cholesterol – Triacylglycerols/2.2 – HDL-cholesterol

Atherogenic index was calculated as ratio of LDL-cholesterol to HDL-cholesterol. Asymmetric dimethyl-arginine (ADMA) concentrations in plasma were measured by ELISA test (DLD Diagnostika GmbH, Hamburg, Germany). Blood elements were detected by hematological autoanalyzer Sysmex KX 21 (Mundelein, IL, USA).

Intakes of vitamins, mineral and trace elements only in natural form were considered (including biscuit consumption). Consumption of nutritional or pharmaceutical additives was excluded. The study was carried out in late autumn and winter. Pair t-test was used on comparison between results before consumption of biscuit, after 2-month consumption and after 2-month wash-out, other between results after consumption and 2-month wash-out. P values less than 0.05 were considered to indicate statistical significance. The results are expressed as mean ± SEM.

RESULTS

The average body mass index (BMI) of examined group of subjects before biscuit consumption was in range of overweight. WHR (whist hip ratio) presented the same distribution of fat in peripheral and central areas. Blood pressure and body fat were in normal values (Table 1). Lipid parameters before consumption of biscuit were also in normal concentrations. The concentrations of other basic biochemical parameters were also found in reference range: glucose, albumin, calcium, magnesium, potassium, iron as well as liver enzyme activities (amylase, AST, ALT, ALP), total bilirubin and blood elements (leukocytes, erythrocytes, platelets, hemoglobin). These values showed that the group of volunteers of general population was optimally selected. Data from questionnaires confirmed this fact because intake of macronutrients and micronutrients in all volunteers was in recommended range.

Consumption of biscuit had no effect on antioxidant vitamin plasma concentrations (Table 3) as a consequence of non-changed nutritional habit during biscuit consumption. The significant increase of biscuit compounds (catechins from green tea and selenium from selenized onion) was observed in blood of probands after consumption of biscuit (Table 3). Two months after exclusion of consumption, the values derived from biscuit tended to return to their level before consumption. Other biscuit compounds (quercetin from onion and curcumin from curcuma) were not measured.

The significantly reduced values of DNA strand breaks, DNA breaks with oxidized purines as well as oxidized pyrimidines (Table 4) were found after two month biscuit consumption. Two months after biscuit exclusion a trend to return of lymphocyte DNA damage values to levels before consumption was observed.

DISCUSSION

Health effects of consumption of four bioactive compounds obtained in biscuit are introduced in literature by their solo consumption. The greatest group of polyphenols are flavonoids, from which some have multiple stronger antioxidant effects in comparison to vitamin C and E [10]. Flavonoids exhibit biological activities, including antioxidative, antiallergic, antiinflammatory, antiviral, antitumor, and vasodilating actions. Tea is one of the richest sources of flavonoids, in particular catechins. The most important catechins are: epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate. The last named indicates the highest biological effects such as antioxidative, antimutagenic, anticarcinogenic. An antioxidant activity of epigallocatechin-3-gallate exceeds vitamin C effect at least 100-fold [11].

Table 3. Selected cardiovascular risk factors and concentrations of antioxidant vitamins, catechins and selenium

Parameters	Before consumption (1)	After 2-month consumption (2)	After 2-month wash-out (3)
Total cholesterol (mmol/l)	5.17 ± 0.14	4.96 ± 0.13*	5.14 ± 0.13^
LDL-cholesterol (mmol/l)	3.18 ± 0.12	2.9 ± 0.11**	3.18 ± 0.12^^
Atherogenic index	2.64 ± 0.13	2.38 ± 0.12*	3.18 ± 0.12***,^^^
Asymmetric dimethyl-arginine (nmol/ml)	1 ± 0.03	0.46 ± 0.03***	0.87 ± 0.02**,^^
Vitamin C (µmol/l)	24.3 ± 1.6	26 ± 1.4	23.7 ± 1.3
Vitamin E (µmol/l)	29 ± 0.7	30.6 ± 0.9	31.1 ± 1.2
Beta-carotene (µmol/l)	0.85 ± 0.06	0.84 ± 0.07	0.88 ± 0.06
Lycopene(µmol/l)	0.66 ± 0.04	0.65 ± 0.05	0.59 ± 0.01
Epicatechin (µg/l)	6.36 ± 0.49	10.36 ± 0.75***	8.51 ± 0.69*,^
Epigallocatechin gallate(µg/l)	35.3 ± 1.5	53 ± 1.9***	45.1 ± 2.2***,^^
Epicatechin gallate (µg/l)	21 6 ± 1	30.2 ± 1.5***	25.5 ± 1.3**,^
Selenium (µg/l)	79.1 ± 1.7	90.5 ± 1.4***	81.7 ± 1.5*,^^^

*(2), (3) *vs* (1); *P < 0.05, **P < 0.01, ***P < 0.001 - ^(3) *vs* (2); ^P <0.05, ^^P < 0.01, ^^^P < 0.001

Table 4. Products of DNA damage

Parameters	Before consumption (1)	After 2-month consumption (2)	After 2- month wash-out (3)
DNA breaks (AU)	84.4 ± 2.7	50.4 ± 2***	67.9 ± 2.8***,^^^
DNA breaks + oxidized purines (AU)	159 ± 3	99 ± 3***	123 + 4***,^^^
DNA breaks + oxidized pyrimidines (AU)	158 ± 4	119 ± 3***	138 ± 3***,^^^

*(2), (3) *vs* (1); ***P < 0.001 - ^(3) *vs* (2); ^^^P < 0.001

High content of polyphenols was observed in curcuma; strong antioxidant properties were found in curcumin. Alcoholic curcuma extract has antiinflammatory, immunomodulative, anticarcinogenic and antisclerotic effects [12]. Oral administration of hydroalcoholic extract of curcuma decreases significantly the LDL and apoB and increases the HDL and apoA in healthy subjects [13]. Pronounced antioxidative properties of the polyphenolic flavonoid quercetin were also described. In an experimental study on diabetic mice significantly decreased levels of DNA damage in peripheral lymphocytes after intraperitoneal dose of quercetin were observed [14]. Quercetin is an inactivator of lipid peroxide radical [15]. Increased selenium intake can decrease cardiovascular risk by reduction of lipid peroxidation, inhibition of inflammation and improving blood lipid spectrum together with antiatherogenic fatty acids [16].

The present literature data about health effects of consumption of individual polyphenols and selenium were proved in our study by consumption of cereal biscuit containing all introduced protective compounds together in one complex. The antiatherosclerotic, antioxidative and anticarcinogenic effects of cereal biscuit consumption were documented by significantly reduced markers of cardiovascular risk as well as by significantly reduced values of DNA strand breaks, DNA breaks with oxidized purines as well as pyrimidines. Selenium together with antiatherogenic fatty acids in biscuit (oleic acid, PUFA, linen seeds) and other antisclerotic biscuit compounds participated on the improvement of lipid spectrum by decrease of total and LDL-cholesterol as well as atherogenic index LDL/HDL (Table 3). ADMA concentration was also significantly decreased by biscuit consumption (Table 3).

The significantly increased plasma concentrations of biscuit protective compounds, *i.e.* catechins, selenium and non-measured but present quercetin and curcumin (plasma concentrations of antioxidative vitamins before and after biscuit consumption were non-changed) suggest that actually the biscuit compounds may prevent from DNA damage and oxidative stress and decrease cardiovascular risk parameters. The biscuit protective compounds decreased the values of DNA strand breaks by 40%, DNA breaks with oxidized purines by 37% and DNA breaks with oxidized pyrimidines by 24%. A stronger protective effect against DNA damage by biscuit consumption with four effective compounds together was apparent if compared these findings with our previous results in vegetarians with dominant plant consumption (lacto-

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ovo-vegetarians) [17]. In that study, the group of vegetarians *vs* non-vegetarians (traditional mixed diet) non-changed values of DNA breaks were found, values of DNA breaks with oxidized purines were decreased only by 16% and values of DNA breaks with oxidized pyrimidines by 18%. This comparison suggests that consumption of several connected bioactive compounds in purposeful produced biscuit provides the stronger protective effect against DNA damage than current plant consumption in vegetarians.

Protective effects of two bioactive compounds (quercetin and catechins) together against DNA damage were described elsewhere. Effects of catechin, epicatechin and quercetin against N-nitrosamines-induced DNA damage were demonstrated in human hepatoma cells. Catechin in the lowest concentration showed the maximum reduction of DNA strand breaks by 23%, the formation of oxidized pyrimidines by 19-21% and oxidized purines by 28-40%. Epicatechin also decreased DNA breaks by 20% and values of oxidized pyrimidines/purines by 33-39%. Quercetin reduced the N-nitrosopiperidine induced formation of oxidized pyrimidines and purines by 17-20% [18]. In another work the protective effects of introduced polyphenols against DNA damage induced by three carcinogenic compounds which occur in the environment were described [19]. In experimental studies on rats the protective effects of quercetin against mercury or nicotine induced DNA damage were found [20, 21]. Regular intake of catechins from green tea drinking might protect smokers from oxidative damages and could reduce cancer risk or other diseases caused by free radicals associated with smoking [22].

In conclusion, the functional food (cereal biscuit with bioactive complex of selenium in organic form, quercetin, curcumin, and catechins presented in natural plant sources), selenized onion, curcuma and green tea consumed during two months significantly decreased the values of DNA damage products (DNA strand breaks, DNA strand breaks with oxidized purines, and pyrimidines). The decrease of parameters was more pronounced than it was by solo consumption of individual compounds (literature data) or by dominant but not very high (common for alternative nutrition) consumption of plant food in vegetarians. These findings as well as other results (significantly reduced concentrations of total cholesterol, LDL-cholesterol, ADMA and value of atherogenic index) suggest that cereal biscuit with antiatherogenic, antioxidative and anticarcinogenic properties may be the correct way for production of similar functional foods in order to provide disease prevention.

The novelty of this study: in harmony with long-term knowledge and experience, selenization of onion (production of selenium in organic form), production of cereal biscuit containing four bioactive compounds together, favourably influenced decreases in DNA damage as well as cardiovascular risk parameters more pronounced than in vegetarians with dominant plant food consumption.

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COMPETING INTERESTS

None

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