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Research paper

Is professional prescription of a commercially derived dietary supplement in colectomysed patients necessary?

Anna Blázovics[a], Laszlo Kursinszki[a], Nora Papp[b], Dezso Kleiner[a], Edit Szőke[a], Gabriella Hegyi[c,*], Anna Szilvás[d]

[a] Department of Pharmacognosy, Semmelweis University, Üllöi rd. 26, 1085 Budapest, Hungary
[b] Department of Applied Chemistry, Faculty of Food Science, Corvinus University of Budapest, Villányi St. 29-43, 1118 Budapest, Hungary
[c] Department of Complementer Medicine, University of Pécs, Faculty of Medicine, Vörösmarty St. 4, 7622 Pécs, Hungary
[d] Department of Gastroenterology, Saint John Hospital, Diós árok 1-3, 1125 Budapest, Hungary

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ABSTRACT

Introduction: Colorectal cancers are the second most common cause of mortality in the USA, Western Europe and also Hungary. After the removal of a tumor, cancer predisposition still remains, therefore secondary prevention is needed. Several reports are known about the effects of nutritional supplements in the improvement of patient life quality. Therefore the question arises whether natural food supplement products with several bioactive agents can modify immune reactivity or influence redox homeostasis.

Methods: In the present study we report on the negative output of the commercially derived dietary supplement "Vegetable and fruit color compound concentrates OÉTI 45/É sample" in colectomysed patients. Quantitative analysis of anthocyanins and flavonoids in this dietary supplement, was carried out using HPLC-DAD-ESI–MS/MS. Caucasian volunteers ($N=26$) were treated with the dietary supplement for 3 months 5–10 years after their colectomy. The healthy control volunteers ($N=10$) of both genders received the supplement the same way. The dose was 2×3 g/day. Ultrasonography, routine laboratory tests (30 parameters), tumor marker (AFP, CEA, CA19-9, PSA) determinations and redox parameters (SOD, GSHPx, HbA1c, reducing power, chemiluminescence intensity) were carried out for each person.

Results: Diet-related bioactive compounds were intensively examined in vitro, although in vivo and in vitro effects are different and the mode of actions in vivo are not yet known in detail. Tentative identification of dietary supplements based on mass spectrometry data for published constituents of plants, twenty anthocyanins and flavonoid glycosides were identified.

Several routine laboratory parameters were not altered after treatment compared to start time data in colectomysed patients and control volunteers. The treatment did not change the tumor marker levels significantly, but in each case the values were elevated in examined groups. Redox parameters of colectomysed patients showed strengthened free radical reactions, namely rebound effect against antioxidant excess, although tendency was observed in controls as well. Anti-inflammatory effects of the natural product was not clearly observed.

Abbreviations: AFP, alpha-fetoprotein; Akt, serine/threonine kinase (protein kinase B); ALT, alanine aminotransferase; AST, aspartate aminotransferase; CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CL, chemiluminescence; CRP, C-reactive protein; cSrc, a member of the Src protein tyrosine kinase family; ERK1/2, extracellular-signal-regulated kinase 1/2; eNOS, *nitric* oxide synthetase; ETS, (E-twenty six) family of transcription factors; GGT, gamma-glutamyl-transpeptidase; GSH, Pxphospholipid hydroperoxide glutathione peroxidase; HbA1c, glycosylated hemoglobin; HIF-1, hypoxia-inducible factor-1; INR, international normalized ratio (coagulation); LDH, *lactate dehydrogenase*; MAPK, mitogen activated protein kinase (serine/threonine kinase); MCH, mean cellular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MMP-s, matrix metalloproteinases; MPV, mean platelet volume; NAD(P)H, nicotinamide adenine dinucleotide phosphate reduced form; NF-κB, nuclear transcription factor κB; PAI-1, plasminogen activator inhibitor-1; p38, mitogen-activated protein kinase; p53, tumor protein 53 (tumor suppressor); PSA, prostate-specific antigen; REF-1, redox factor-1; RDW, red cell distribution width; ROS, reactive oxygen species; SOD, superoxide dismutase; src, oncogene (cytoplasmic tyrosine kinase); uPA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2.

* Corresponding author. Fax: +36 12813035.
E-mail addresses: drhegyi@hu.inter.net, yamamoto@yamamoto.hu (G. Hegyi).

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Conclusion: Today, there are countless products on the market, thus experts in healthcare, doctors, pharmacists and dieticians should have adequate knowledge of dietary supplements, and how can they become important tools for a healthy lifestyle.
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1. Introduction

Thanks to wide ranging research, approximately half of the defined 200 tumor types can be cured. In recent decades, many new excellent special therapies have come to light in cancer research, although in spite of enormous effort the chances of definitive recovery is very different for various tumors [1]. Therefore again and again the researchers turn to nutrition for secondary prevention [2–5].

The roles of $O_2^{\bullet -}$ and NO free radicals or H_2O_2 are significant in the process of angiogenesis. Reactive oxygen species (ROS) generating NAD(P)H oxidases contribute to redox-activation signaling and take part in the angiogenesis [6].

Unfavorable changes of redox-homeostasis generate cytokine and free radical overflow. These molecules influence many intracellular signaling pathways in different diseases as well as in cancerous processes [7].

Low concentrations of free radicals stimulate the proliferation of endothelial cells, because ROS may modulate various kinases or may activate transcription factors directly to effect gene regulation in the nucleus. HIF1-α, REF-1, p53, NF-κB, ETS redox-sensitive transcription factors are involved in angiogenesis. cSrc, Akt, eNOS, p38, MAPK, ERK1/2 are the main signal routes in which free radicals have a modulating role. VEGF, MMP-s uPA and PAI-1 are results of redox sensitive gene expression [1,8–11].

Previous publications have strengthened the fact that the VEGF VEGFR2, ROS, NAD(P)H oxidases are potential therapeutic targets for tumor angiogenesis as well as the effectiveness of several polyphenols, flavonoids, catechines and stilbenes for their inhibitions during the angiogenesis [12,13].

Since the physiological background remains largely unknown, especially the effects of special nutrition supplement in secondary prevention, our question was: “How can the polyphenol- and vitamin-reach products protect the patients from serious metastases?” We wanted to know, whether the applied vegetable and fruit component composition can be a suitable food supplement for patients who had been operated on for colon cancer. Could we follow the effectiveness of operation or therapy using an earlier-developed simple routine method of luminol-dependent chemiluminesce and enzymatic and measurements of non-enzymatic antioxidant parameters?

2. Materials and methods

2.1. Reagents

Luminol, hydrogen peroxide and microperoxidase were obtained from SIGMA (St. Louis). Tumor marker CEA, CA19-9, AFP and PSA kits (LIA-mAT immunoluminometry) were obtained from LIA-mAT (Budapest) and HbA1c-kit from BioRad (Budapest). CRP (CRP/AUT-000) was obtained from Diagnosticum Ltd. GSHPx and SOD kits were purchased from RANDOX (England). All other reagents in analytical grade were purchased from Reanal (Budapest).

“Vegetable and fruit color compound concentrates” diet supplement permission number: OÉTI 45/É was commercially available in Hungary. Declared component composition by manufacturer: *Sambucus nigra* (berry), *Vaccinum mirtillus* (extract), *Rubus nigra* (berry), *Beta vulgaris var. rubra* (tuber), *Vitis vinifera* (berry), *Hippophae rhamnoides* (fruit oil), *Hippophae rhamnoides* (juice), *Hippophae rhamnoides* (berry juice), *Aronia rotundifolia* (extract), *Hibiscus sabdariffa* (extract)

Antioxidant content per 100 g of dietary supplement: total polyphenols: 2100 mg, from this anthocyanidins 330 mg and flavonoids 30 mg, vitamin C is 48 mg and carotenoids are 2.2 mg.

3. Qualitative analysis of anthocyanins and flavonoids in the dietary supplement by HPLC-DAD-ESI–MS/MS

3.1. Sample preparation

Dietary supplement samples (10 g) were extracted with 10.00 mL 70% aqueous methanol containing 1% formic acid by sonication at 27 ± 2 °C (Braun Labsonic U, Melsungen, Germany) for 10 min. The extracts were centrifuged (6000 rpm, 2500 g, 10 min). The supernatants were concentrated under vacuum to dryness at 35 °C. The residues were dissolved in 50% aqueous methanol containing 0.1% formic acid and were filtered on Minisart RC 15 (0.2 μm) syringe filter before analysis.

4. HPLC-DAD-ESI–MS/MS

High-performance liquid chromatography with diode array detection coupled to electrospray ionization and triple quadrupole mass spectrometry is an indispensable tool for the characterization of phenolic compounds in fruit juices [14].

For chromatographic separation and mass spectral analysis, an Agilent 1100HPLC system (degasser, binary gradient pump, autosampler, column thermostat and diode array detector) was used coupled with an Agilent 6410 Triple Quad LC/MS system equipped with ESI ion source (Agilent Technologies, Palo Alto, CA).

HPLC separation was achieved on a Zorbax Eclipse XDB-C18 reversed-phase column (150 × 4.6 mm i.d.; 5 μm particle size, Agilent, Santa Clara, CA, USA), with a C18 guard-column (4 mm × 3 mm i.d., 5 μm particle size, Phenomenex, Torrance, CA, USA). Gradient elution was applied with methanol (A) and 2.5% formic acid (B) as solvents. Gradient program: 0.00 min, 18% A; 30.00 min, 45% A; 34.00 min, 60% A; 37.00 min, 60% A; 40 min, 18% A; 50 min 18% A. The solvent flow rate was 1.00 mL/min and column temperature was set at 25 °C. The injection volume was 10 μl. By solvent splitting, 30 % eluent was allowed to flow into the mass spectrometer [15].

Electrospray conditions were as follows: drying gas (N_2) temperature, 350 °C; flow rate, 9 L min^{-1}; nebulizer pressure, 45 psi (N_2); fragmentor voltage, 40 V for anthocyanins and 100 V for flavonoids; capillary voltage, 3500 V. High purity nitrogen was used as collision gas and collision energy was changed between 5 and 40 V according to differences in molecule structures. Full scan mass spectra were recorded both in negative and positive ion modes over an *m/z* range of 50–1000. Both precursor ion and product ion analysis techniques were applied and the former was especially useful for detection of minor components [16]. Peaks were identified by comparing their retention time, molecular ion ($[M]^+$ or $[M-H]^-$) and fragment ions with standards or published data, and by analysing information on their occurrence in the plant sources.