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Abstracts

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Aim: The aim of this study was to assess the bioavailability of a functional food (FF) with a high content of natural antioxidants (tomato carotenoids and virgin olive oil polyphenols) to be used as adjuvant therapy in CHC antiviral treatment.

Subjects and Methods: The bioavailability study was performed in 8 healthy volunteers (5 treated and 3 controls) and in 40 patients with CHC (20 treated and 20 controls) subjected to antiviral therapy. Healthy subjects and patients consumed 100 g of the FF (23 mg of carotenoids/die) respectively for one week and for one months. The measure of carotenoid seric levels (β-carotene, lutein, lycopene all-trans) and vitamin E was performed by HPLC method.

Results: The percentage of variation of antioxidant compounds before and after supplementation with FF are reported in the table. The increase in the carotenoid concentration was higher in healthy subjects respect to CHC treated patients, while the CHC-control patients underwent to a dramatic decrease of carotenoid concentration.

	Healthy volunteers		CHC patients	
	treated, %	controls, %	treated, %	controls, %
Lycopene all-trans	213	−2	47	−40
Lycopene 5-cis	262	−3	79	n.d.*
β-carotene	133	4	53	−32
Lutein	120	1	7	−32
Vitamin E	23	2	57	−36

*Not detected – below the limit of detection.

Conclusion: Tomato carotenoids present in our FF are highly bioavailable. The supplementation with FF reverted the depletion of these compounds observed in patients with CHC during therapy treatment.

PS.Q40

An *ex vivo/in vitro* Human Model of Osteoclastogenesis to Screen Osteoclastogenic Compounds and to Study Osteoclast Interactions with Different Primary Cultures

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Currently, no human test systems are available to investigate the osteoclastogenic effects of substances that epidemiological studies and/or animal and in vitro studies suggest might have a beneficial effect on bone health. A possibility to develop such a system has been recently provided by the development of an ex vivo/in vitro model of osteoclastogenesis. The discovery of the hemopoietic origins of the osteoclasts as well as the identification of the osteoclast differentiation factor, RANKL, give the opportunity to generate them starting from the human peripheral blood mononuclear cells (PBMCs) and also to culture PBMC in absence of the stromal/osteoblastic cells [1, 2]. Further, the precise characterization of the osteoclast precursors allowed to select almost pure precursors from the peripheral blood through the isolation of the CD14-positive and M-CSF-dependent monocytes [2, 3]. Finally, the possibility to analyze bone resorption by culturing osteoclast cells on dentine slices (pit resorption assay) allow to employ such a system as 'a functional biomarker' through the measurement of the resorption area as an index of the osteoclasts functionality.

We set up such a model (a) to use it as a screening method to investigate the effect of pure compounds on human osteoclast differentiation; (b) to study the level of total exposure to dietary substances with osteoclastogenic activity; and (c) to have a controlled system to evaluate the interaction and the influence of different primary cell cultures on human osteoclastogenesis (co-cultures).

References

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PS.Q41

Angiotensin I-Converting Enzyme Inhibitory Peptides Derived from *Chlorella*

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Chlorella, a freshwater unicellular green alga, has become a popular foodstuff worldwide. Administration of this alga has been shown to have a variety of physiological functions, such as enhancing the growth rate of animals, ameliorating the blood glucose and lipid level, boosting the immune function, and preventing the raise of blood pressure in human and test animals. Recently, we found that peptides from pepsin-hydrolysates of *Chlorella* (strain KP-3001) exhibited rather high activity in inhibiting angiotensin I converting enzyme (ACE), which catalyzes the formation of angiotensin II, a strong vasopressor. Further separation and purification of ACE-inhibiting peptides from hydrolysates were done by gel filtration and HPLC. Rather low IC_{50} values, the peptide concentration resulting in 50% inhibition of ACE activity, were obtained for the partially purified fractions after gel filtration. The degree of hydrolysis, the composition and the sequence of amino acids of the purified peptides were analyzed. Bioassay for measuring the acute as well as chronic toxicity of these peptides was conducted. The action of these peptides was elucidated by hypertensive animal model.

PS.A18

Inhibition of Xanthine Oxidase by Chlorella and the Water Extract

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Chlorella, a type of unicellular green algae, contains essential amino acids, minerals, and fibers. Administration of *Chlorella* has been shown to play some biochemical functions, such as promoting the growth rate of animals, ameliorating blood glucose and lipids in animals, boosting immune function, preventing stress-induced ulcer, and influencing oxidative stress in ethionine treated rats. Thus, the aims of this study were to examine anti-oxidative effects of *Chlorella* by measuring the conversion of xanthine to uric acid and superoxide through the effects of xanthine oxidase.

Bovine milk xanthine oxidase (1 mU/mL) was incubated with 15 mM xanthine in the presence and absence of the test compounds at 25°C. Uric acid formation was determined by absorbance at 290 nm. The initial rate was calculated from the linear portion of each reaction, from 0.5 to 2.5 min in most cases.

Chlorella (80 mg/ml) produced slight inhibition on uric acid production (36.3%), however, the aqueous *Chlorella* extract, dose between 5 and 40 mg/ml, produced inhibition rate over 50%. Allopurinol (50 μg/ml), a known xanthine oxidase inhibitor and used as a positive control, produced an inhibitory rate about 45%. Further studies are to determine the effective component of *Chlorella* and its IC_{50} in inhibiting xanthine oxidase.

PS.A19

The 10 Basic Requirements for a Scientific Paper Reporting Antioxidant, Antimutagenic or Anticarcinogenic Potential of Test Substances *in vitro* Experiments and Animal Studies *in vivo*

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There is increasing evidence that chemicals/test substances cannot only have adverse effects, but that there are many substances that can (also) have a beneficial effect on health. Food and Chemical Toxicology regularly publishes papers in this area. Since the journal has every intention in continuing to do so in the near future, it has become essential that studies reported reflect an adequate level of scientific scrutiny. Therefore a set of essential characteristics of studies has been defined. These basic requirements are default properties rather than non-negotiable: deviations are possible and useful, provided they can be justified on scientific grounds. The 10 basic requirements for a scientific paper reporting antioxidant, antimutagenic or anticarcinogenic potential of test substances in vitro experiments and animal studies in vivo concern the following areas: (1) Hypothesis-driven study design, (2) The nature of the test substance, (3) Valid and invalid test systems, (4) The selection of dose levels and gender, (5) Reversal of the effects induced by oxidants, carcinogens and mutagens, (6) Route of administration, (7) Number and validity of test variables, (8) Repeatability and reproducibility, (9) Statistics, and (10) Quality Assurance.

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PS.A20

Evidence for Formation of (3′-Dehydro)-Lutein from Both Lutein and Zeaxanthin in Humans

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Background and Objective: The macular pigments lutein and zeaxanthin have both been hypothesised to reduce the risk of age-related macular degeneration. Because pharmacokinetic information on these xanthophylls and their metabolites is still very limited, we have investigated their plasma kinetics by multiple dosing studies in healthy volunteers.

Design: The plasma concentration response to lutein and zeaxanthin intake at two dosages each was investigated in open label, parallel group studies. Lutein was dosed at 4.1 and 20.5 mg per day in capsules (Lutein 5% TG, Roche Vitamins) and dosing groups included 8 subjects per group (4 volunteers of each gender). In the zeaxanthin trial, doses of 1 and 10 mg (Zeaxanthin 5% TG, Roche Vitamins) per day were administered to 10 subjects per dosing group (5 volunteers of each gender). After a 7 days run-in period subjects were dosed with either lutein or zeaxanthin for 42 consecutive days (dosing period). Plasma concentration time profiles were monitored over the dosing period and 25 or 34 days post-dosing in the lutein and zeaxanthin study, respectively.

Results: Average baseline concentrations were 0.17 and 0.048 μmol/L for lutein and zeaxanthin, respectively. Concentrations increased during the dosing period, reaching plateau levels. Steady state concentrations at day 42 were 0.59 and 1.64 μmol/L for lutein and 0.20 and 0.92 μmol/L for zeaxanthin for lower and higher doses, respectively. Efficient half-life was similar for lutein and zeaxanthin and was approximately 5 to 7 days. In both the lutein and the zeaxanthin trials, plasma concentrations of 3′-dehydro-lutein increased in parallel with xanthophyll concentrations during the dosing period, and decreased post-dosing. Steady state xanthophyll concentrations were linearly related with the corresponding 3′-dehydro-lutein levels. Formation of 3′-dehydro-lutein from each of the xanthophylls was supported by kinetic modelling.

Conclusion: The studies provide strong evidence for formation of 3′-dehydro-lutein form both lutein and zeaxanthin.