ABSTRACTS

International Symposium on the Role of Tomato Products and Carotenoids in Disease Prevention

April 10, 2001
New York, New York

1535-3702/02/22710-00000928$15.00

SINGLE DOSE PHARMACOKINETICS OF TOMATO-BASED LYCOPENE IN HEALTHY MEN. [1]Diwadkar-Navsariwala, V., [2]Gustin, D.M., [3]Rodvold, K.A., [2]Sosman, J.A., [1]Stacewicz-Sapuntzakis, M., [2]Murray, J.L., [2]Tiller, P.A., and [1]Bowen, P.E., [1]Department of Human Nutrition and Dietetics, [2]Department of Hematology/Oncology [3]Department of Pharmacy Practice. University of Illinois at Chicago, Chicago, IL USA

The purpose of this study was to evaluate the pharmacokinetics of lycopene delivered as a tomato juice formulation. Twenty five (5 per dose) healthy male subjects received a single lycopene dose of 10, 30, 60, 90 and 120 mg. Blood was collected before administration of the lycopene dose (0 hour) and 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48, 72, 96, 168, 240, 336, 408, 504, 576, 672 hours thereafter. Serum and chylomicron concentrations of lycopene (cis/trans isomers) were measured by HPLC analysis. Subjects were instructed to adhere to a low carotenoid diet during the study. Noncompartmental pharmacokinetic parameters were determined after subtracting the baseline (0 hour) lycopene concentration.

Values of the area-under-the-curve (AUC) and maximum serum concentration (C_{max}) were not proportional with changes in dose. Overall, the observed AUC and C_{max} values were similar at dose levels of 30 mg and 60 mg, and at 90 mg and 120 mg. These data suggest saturation of lycopene absorptive mechanisms or, less likely, increased plasma clearance with very high consumption of tomato products. The percentage (%) of trans isomer increased quickly and mean ($\pm$SD) time (T_{max}) to reach C_{max} was 12.9 $\pm$ 8.2 hrs. Serum concentrations of trans-lycopene were observed to decrease with time thereafter. The proportion of cis and trans isomers was not dose dependent. At baseline, average % of trans-lycopene in the serum was 32.8 $\pm$ 4.6. At C_{max} the average % trans was 43.9 $\pm$ 4.4. The prevalence of trans isomers in the beverage (85 %) was reflected in the early rise of trans isomers in the serum. These results have important implications for planning clinical trials of multiple-doses of lycopene in cancer chemoprevention.

ABSORPTION AND METABOLISM OF SYNTHETIC AND NATURAL (TOMATO) DEUTERATED LYCOPENE. [1]Ferreira, A.L.A., [2]Tang, G., [3]Grusak, M.A., [2]Qin, J., [2]Dolnikowski, G.G., [2]Yeum, K-J, [2]Krinsky, N.I., and [2]Russell, R.M. Fac. Medicina UNESP, CP 584, Botucatu, SP, 18618-970, Brazil. [2]Jean Mayer USDA-Human Nutrition Research Center on Aging at Tufts University, Boston, MA, 02111. [3] USDA-ARS Children's Nutrition Research Center, Houston, TX 77030

To compare the bioavailability of lycopene from tomatoes to that of synthetic lycopene, we carried out a pilot study using tomatoes grown in 25% D_2O as the source of lycopene and an advanced LC/APCI (atmospheric pressure chemical ionization)-MS method to analyze the blood samples from the subjects. Two subjects (1 male & 1 female) ate hydroponically grown tomatoes containing deuterium- enriched lycopene (enrichment peak at M +10) and two subjects (1 male &1 female) ate synthetic D10 lycopene (6 mg in corn oil). The tomatoes were steamed and pureed, and contained 8.9 mg and 9.5 mg deuterated lycopene per dose (80 – 84 g wet weight). The detection limit of D_{10} lycopene using LC/APCI-MS method is 1 ng. Our results showed that up to 36 days after taking an oral lycopene dose (synthetic or tomato) with a liquid formula drink (35% fat), the area under the curve of the average serum percent enrichment response of labeled lycopene from the synthetic lycopene dose was 23.1 $\pm$ 9.2 (%•day/mg) and that of labeled lycopene from the tomato dose was 8.3 $\pm$ 1.9 (%•day/mg). Thus, the bioavailability of synthetic lycopene appears to be almost three times higher than that of lycopene from cooked and pureed tomatoes.

Using a purified post-mitochondrial fraction of rat intestinal mucosa, we investigated the oxidative cleavage products of lycopene metabolism *in vitro*. The enzymatic preparations were supplemented with lipoxidase (soybean), NAD^+, KCl and DTT and the products were separated using HPLC, analyzed by UV/Vis spectrophotometry, and the molecular weights of the products was analyzed by APCI-MS. The incubations produced two groups of products: cleavage products and oxidation products. The likely cleavage products are: (1) lycopene-3-one, apo-13-one with λmax = 365 nm and m/z = 272; (2) 15,15'-lycopenal with λmax = 380 nm and m/z = 284; (3) 2-apo-5,8-epoxy-lycopene with λmax = 415, 435, 470 nm and m/z = 510. The likely oxidative metabolites are: (4) 5,6-,5',6'-diepoxy-lycopene with λmax = 410, 440, 465 nm and m/z = 568; (5) 5,8-lycopene furanoxide (I) with λmax = 410, 440, 465 nm and m/z = 552; (6) 5,8- lycopene epoxide (II) with λmax = 410, 435, 465 nm and m/z = 552; (7) ε-3-one-5,8 lycopene furanoxide with λmax = 400, 420, 450 nm and m/z = 566. These results demonstrate that both central and excentric cleavage of lycopene occurs in the rat intestinal mucosa. (Supported in part by a grant from Fundunesp, USDA ARS, and BASF).

SERUM CAROTENOID CONCENTRATIONS AMONG US CHILDREN. Ford, E.S., Gillespie, C., Ballew, C., Sowell, A. Centers for Disease Control and Prevention, Atlanta, GA, USA.

Carotenoids, a class of phytonutrients with important antioxidant properties, may affect the risk of several chronic conditions. Little is known about carotenoid concentrations in US children. Using data from the National Health and Nutrition Examination Survey III (1988-1994), we examined the distributions of serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene measured by high performance liquid chromatography among 4,231 US children aged 6-16 years. Boys had slightly higher mean and median concentrations of all carotenoid concentrations than girls but the differences were not significant. Age was inversely related to all carotenoid concentrations ($p \leq 0.001$) except lycopene ($p=0.584$). African American children had the highest mean and median and white children the lowest serum total carotenoid concentrations ($p<0.001$). African American children had the highest mean and median concentrations of lutein/zeaxanthin and lycopene, Mexican American children had the highest concentrations of α-carotene and β-cryptoxanthin, and white children had the highest β-carotene concentration although race or ethnicity was not associated with β-carotene concentration. Poverty income index was positively associated with α-carotene ($p=0.005$) and β-carotene ($p=0.022$) concentrations and inversely with lutein/zeaxanthin ($p=0.018$) concentrations. Body mass index percentiles were inversely associated with all carotenoid concentrations ($p \leq 0.001$) except lycopene. These data show significant variations in serum carotenoid concentrations among US children. To the extent that the antioxidant and other properties of carotenoids confer health benefits, overweight children and children from poor families may increase their risk for excess morbidity and mortality from future chronic diseases.

IN-VIVO RESONANT RAMAN DETECTION OF CAROTENOID ANTIOXIDANTS IN HUMAN TISSUE, Gellermann, W., Ermakov, I.V., and McClane, R.W. Department of Physics, University of Utah, Salt Lake City, Utah

Carotenoids are powerful antioxidants playing an important role in the body's defense against the effects of reactive oxygen species and free radicals. Studies have shown an inverse correlation between high dietary intake of carotenoids and the risk of various cancers, cardiovascular disease and degenerative diseases. We have developed a new non-invasive optical technique based on Raman Spectroscopy to measure carotenoid levels in human tissues including the retina, skin, oral cavity and other tissues of volunteers. A portable instrument measuring skin carotenoid levels will be demonstrated at the poster presentation.

In the case of human skin, the five most concentrated carotenoids are lycopene, alpha-carotene, beta-carotene, phytoene, and phytofluene, accounting for 60-70% of total carotenoid content. These molecules play an important role in the skin's antioxidant defense system acting as scavengers for free radicals, singlet oxygen, and other harmful reactive free oxygen species, which are formed by excessive exposure of skin to sun light, for example. If unbalanced by the carotenoids and other antioxidants, the effects of reactive oxygen species can lead to premature skin aging, to oxidative cell damage, and even to the formation of skin cancers. In animal models carotenoids have been shown to inhibit carcinoma formation in the skin. Carotenoids have also been studied as inhibitors of a variety of other cancers and pre-cancers, including sustained remissions in oral leukoplakia patients. In the retina, studies have shown an inverse correlation between levels of the macular pigments lutein and zeaxanthin, and age-related macular degeneration, a leading cause of blindness.

Carotenoids are □-electron conjugated carbon-chain molecules ($C_{40}H_{56}$) and feature two prominent Raman lines at 1158 and 1528 cm^{-1} under resonant laser excitation. Since the Raman effect is linear, the strength of each line is a measure for the concentration of the carotenoids. We developed several Raman instruments for in vivo measurements of carotenoid concentrations of various tissues. These instruments are suitable for clinical settings. We validated our Raman methodology by comparing the Raman results with the established technique of high performance liquid chromatography in excised tissue samples. These experiments demonstrate excellent linear scaling of the Raman carotenoid peaks with concentration. In our macular pigment detection project, we are able to obtain a carotenoid reading within a fraction of a second at a light exposure level which is about 500 times below the maximum permissible exposure limit. Measurements of carotenoid levels in skin and other tissues can similarly be obtained at safe light levels within a few seconds.

Our results demonstrate that resonance Raman spectroscopy is a highly promising technology for the measurement of tissue carotenoid levels in living humans. It is non-invasive, precise, sensitive, specific, rapid, reproducible, and objective. Our technique can be used for rapid screening of carotenoid levels in large populations and may have applications for assessing antioxidant status and risk for diseases related to oxidative stress. The technology appears well suited to be used for guidance and monitoring of nutritional interventions.

LYCOPENE INHIBITS PROLIFERATION, INDUCES APOPTOSIS, AND DOWNREGULATES CYCLOOXYGENASE-2 GENE EXPRESSION IN NEOPLASTIC RAT MAMMARY EPITHELIAL CELLS. *Kashfi, K. and †Cohen, L.A., * Department of Physiology and Pharmacology, City University of New York Medical School, New York, NY; † Division of Nutrition and Endocrinology, American Health Foundation, Valhalla, NY; ¶ The Rockefeller University, New York, NY; U.S.A.

Lycopene is a carotenoid present in many fruits and vegetables. Epidemiological studies suggest that a high intake of dietary lycopene protects against a variety of cancers. The mechanism(s) for this anticarcinogenic effect is not clearly established. In vitro studies suggest that it may act as an antioxidant and it may also inhibit DNA synthesis. In this study we evaluated other possible mechanisms for this anticarcinogenic effect. We used a well characterized neoplastic rat mammary epithelial cell line derived from N-nitrosomethylurea (NMU)-induced mammary tumors. Our data shows that in the micro-molar (200-800) concentration range, pure lycopene inhibited cell proliferation which was both concentration- and time- dependent. To characterize this further, using flow cytometry we evaluated whether lycopene could induce programmed cell death. Lycopene treatment produced a clear apoptotic population at 24 hrs with the percentage of S phase cells increasing from 38% to 50% at the expense of the G1 population which decreased from 47% to 38%. We also evaluated the effects of lycopene on cyclooxygenase (COX) expression. There are at least two COX activities expressed in humans. COX-1 is constitutively expressed whilst COX-2 is elevated in many different cancers. Base line steady state mRNA levels for COX-2 as determined by RT-PCR was about 3-fold higher than COX-1. Lycopene downregulated COX-2 mRNA levels by about 80% without affecting COX-1 expression. In conclusion, our data proposes some possible mechanisms for the chemopreventive effects of this carotenoid.

TOMATO SAUCE SUPPLEMETATION (TSS) INDUCES APOPTOSIS IN HUMAN PROSTATE CANCER. [1]Kim, H-S, [1]Chen, L., [1]Duncan, C., [2]Ghosh, L., [3]Sharifi, R., [4]Christov, K., [1]Bowen, P. [1]Dept. of Human Nutrition and Dietetics, [2]Dept. of Pathology, [3]Dept. of Clinical Surgery, [4]Dept. of Surgical Oncology, University of Illinois at Chicago, Chicago, IL, USA.

High lycopene intakes have been associated with reduced risk of prostate cancer. Resistance to apoptosis enhances the malignancy of cancer cells. We studied the effect of tomato consumption on apoptosis in hyperplastic and malignant prostate lesions. Thirty two patients diagnosed with adenocarcinoma of the prostate were supplemented with tomato sauce (TSS) pasta entrees (30mg/lycopene) for 3 weeks. Needle biopsy before TSS and resected prostate tissue post TSS were fixed in formalin and embedded in paraffin. Hematoxylin-eosin sections were prepared for assessment of tumor morphology and the grade of invasion [Gleason(GL) classification]. The TUNEL assay was used on parallel sections to identify the apoptotic cells. Paired t-test was used to compare the percentage of apoptotic cells (AI) before and after treatment with TSS. Average age of patients was 63.7±6.1yrs and African Americans predominated (75%). Prostate Specific Antibody (PSA) levels were decreased by TSS (10.9±1.1 vs. 8.7±0.9ng/ml, $p<0.001$). TSS induced apoptotic cell death in prostate hyperplastic lesions (AI= 0.7±0.5% vs. 1.4±1.6%, $p<0.05$) and in carcinoma (AI=0.8±0.7% vs. 2.7±3.0%, $p<0.01$). Induction of apoptosis by TSS showed a tendency to increase in advanced grade tumors (GL3-4 AI=3.0±3.4%) compared to low grade tumors (GL1-2 AI=1.8±2.0%). We conclude that TSS can suppress the growth of human prostate hyperplastic lesions and carcinomas by inducing apoptotic cell death.

(Supported by grant from the National Foundation for Cancer Research)

DOSE-DEPENDENT EFFECT OF LYCOPENE ON HUMAN PROSTATE CANCER CELLS. [1]Kim, L., [1]Rao, A.V., and [2]Rao, L.G. [1] Department of Nutritional Sciences, University of Toronto [2] Calcium Research Laboratory, St. Michael's Hospital, Toronto, Ontario, Canada

Recent epidemiological studies have shown an inverse relationship between consumption and serum lycopene levels and the risk of prostate cancer. The objective of this study was to measure the effect of lycopene on the cell proliferation of LNCaP human prostate cancer cells in culture. The cells were grown for 2 days in medium consisting of RPMI-1640, supplemented with 10% FBS and antibiotic. Lycopene was then added for 24, 48, 72 and 96 hours at different concentrations. A new water-dispersible lycopene in an appropriate vehicle was used for this study. The stock solution of lycopene was diluted in the medium to obtain concentrations of 10^{-6}, 10^{-5} and 10^{-4} M and their corresponding vehicles were diluted in the same manner to be used as controls. The following results were observed: Lycopene at 10^{-6} and 10^{-5} M concentrations significantly reduced the growth of LNCaP cells in a dose dependent manner after 48, 72 and 96 hours of incubation. The inhibitory effect of lycopene on LNCaP cell growth at these concentrations was significantly higher than that of the corresponding vehicle controls. The inhibition by lycopene at 10^{-4} M concentration was not different from that of the vehicle control of the same dilution. Based on these observations, it is concluded that lycopene significantly inhibited the growth of human prostate cancer cells, suggesting an important role for lycopene in human prostate cancer.

EFFECTS OF LYCOPENE ON BENZO(A)PYRENE INDUCED AND SPONTANEOUS MUTAGENESIS IN *LACZ* MOUSE PROSTATE, LUNG AND COLON. [1]Kosinska, W., [1]Chen, M., [2]Cohen, L.A., and [1,3]Guttenplan, J.B. [1]Div. Basic Sciences/Biochemistry, N. Y. U., Dental Center, New York, N Y, [2]Am. Health Found., Valhalla, NY, [3]Dept. of Env.Medicine, N.Y.U. Medical Center, New York, NY

In this study we have evaluated the effects of dietary lycopene on spontaneous and carcinogen-induced mutagenesis in lacZ mice, as consumption of lycopene has been inversely associated with risk for prostate cancer in some epidemiological studies. The mechanism by which lycopene might exert its effects and the stage of carcinogenesis at which it acts are not currently known. We determined the effect of a lycopene-enriched tomato oleoresin (LTO) at 0.5 and 1 mmol/kg diet, on benzo(a)pyrene (BaP)-induced mutagenesis in prostate, lung and colon (n = 6 /group). BaP was administered at five125 mg/kg doses over 12 days via gavage, and mice were euthanized eight weeks later. BaP was significantly mutagenic (4-30X background) in all organs examined. In colon the mutant frequencies (MF's) were: 94.7 $\pm$ 34, 145 $\pm$ 77, and 176 $\pm$ 75 mutants/100,000 pfu in the zero, low and high dose groups of LTO resp. Relative to the BaP group, the increase in MF was significant at the high dose LTO ($p < 0.015$, Mann-Whitney U-test). In lung the MF's were: 26.8 $\pm$ 9.4, 31.7 $\pm$ 13, and 40.3 $\pm$ 12.2 in the zero, low and high dose LTO groups. The increase in MF was significant at the high dose ($p = 0.05$). In contrast, in prostate, mutagenesis was inhibited at both doses. The inhibitory effects were relatively small and not statistically significant, but increased at the higher dose. The values for MF's were: 14.4 $\pm$ 4.1, 13.6 $\pm$ 5.9, and 12.1 $\pm$ 3.0 in the zero, low and high dose LTO groups. This inhibitory effect in prostate repeated in a second study, suggesting it is real. Results in colon also repeated and MF's in lung are still being analyzed. In a long-term (9 month) study on effects of lycopene on the age-related increase in spontaneous mutagenesis, the high, but not the low dose of lycopene reduced mutagenesis in colon from 12.8 $\pm$ 10.3 to 9.3 $\pm$ 2.8, but the difference was not statistically significant and similarly in prostate a nonsignificant reduction in mutagenesis was observed: 6.1 $\pm$ 1.7 to 5.3 $\pm$ 1.2 and again no effect was noted at the lower dose. Results in lung were uninformative because the MF failed to increase with time. Levels of lycopene in plasma of mice in the long-term study (in units of ng/ml plasma) were: 109 $\pm$ 56 and 190 $\pm$ 118 at the low and high dose resp. Lycopene was essentially undetectable in the control group. The results thus far obtained indicate lycopene has different effects in different organs, but it shows a specificity for inhibiting mutagenesis in prostate. The increase in BaP-induced mutagenesis in colon and lung in the lycopene-treated mice could result from prooxidant or cytochrome P-450 induction effects in these organs. This study demonstrates the importance of concentration and organo-specificity in chemoprevention and suggests a specificity for protection by lycopene in the prostate.

SYMPTOM IMPROVEMENT IN *HELICOBACTER PYLORI*-POSITIVE NON-ULCER DYSPEPTIC PATIENTS AFTER TREATMENT WITH THE CAROTENOID ASTAXANTHIN. Lignell A[1], Surace R[2], Böttiger P[1], Borody TJ[2]. [1]AstaCarotene AB, Gustavsberg, Sweden. [2]Centre for Digestive Diseases, Five Dock, Australia.

Helicobacter pylori infection in humans is characterized by a marked infiltration of neutrophilic leukocytes of the gastric mucosa and the generation of reactive oxygen metabolites may play a role in the development of gastritis. Preclinical studies have shown that treatment of *H. pylori* infected mice with astaxanthin-rich algal meal reduced gastric inflammation and bacterial load. The aim with this study was to determine the efficacy of algal meal of the green microalga *Haematoccoccus pluvialis*, rich in astaxanthin, in the treatment of *H. pylori*-positive non-ulcer dyspeptic patients.

An 8-week pilot study was carried out in ten HP-positive non-ulcer volunteers with dyspepia. The patients were enrolled in the study if diagnosed positive for HP on endoscopy. Symptoms were assessed by a gastrointestinal symtom rating scale. Patients were treated with 5 x 8 mg of astaxanthin per day for 21 days. At eight weeks the diagnostic assessments were repeated.

After treatment nine out of ten patients remained HP-positive. On histological examination six patients demonstrated a decrease in the grade of gastritis. Symptom improvement occurred in all patients, especially heartburn and epigastric pain. No treatment-related haematological or clinical abnormalities were observed.

This study indicates that *H. pylori*-positive patients with non-ulcer dyspepsia benefit from treatment with astaxanthin.

BIOLOGICAL ACTIVITY OF TOMATO PHYTONUTRIENTS. Nir Z. and Dov H., LycoRed Natural Products Industries, Ltd., P.O.B. 320, Israel.

The scientific community is unanimous in the belief that free radicals play an important role in the initiation and promotion of degenerative diseases such as atherosclerosis, cancer, cataract and arthritis. Exposure to environmental perils, such as smoking, pollution or irradiation, increases the load of free radicals beyond the ability of the organism to cope with them. The natural mechanism that protects us from free radicals weakens with age. Therefore, the elderly, smokers and those exposed to environmental hazards, are more susceptible to atherosclerosis, cancer and other degenerative diseases.

A growing body of epidemiological evidence associates diets rich in vegetable and fruits with good health and long life. Scientists believe that this is due to the beneficial effect of certain phytochemicals which have antioxidative and free radical neutralizing properties. Leading researchers are conducting investigations in order to trace and evaluate these health-preserving substances.

Recent studies indicate that the tomato is an important source of carotenoids such as Lycopene, Phytoene, Phytofluene and other phytonutrients that are important to our health and well being. Among them, Lycopene receives special attention. Its unique structure that consists of a very long chain of conjugated double bonds, is responsible for its function as a very effective natural antioxidant and a quencher of free radicals.

A growing body of scientific publications based on epidemiological data, cell culture and animal investigations as well as on clinical studies, indicate that it has an important function in the prevention of diseases careful comparison of the bioligical performance of tomato extract to pure Lycopene indicates clearly that the other tomato phytonutrients enhance this ability. This effect was shown in numerous studies that were conducted on Lyc-O-Mato®, the oleoresin extracted from ripe tomatoes.

NEW TOMATO BASED INGREDIENTS. Nir Z. and Dov, H. LycoRed Natural Products Industries Ltd., Beer Sheva, Israel.

Since the discovery of America, the tomato has become an important part of the human diet all over the world. The popularity of the tomato is credited to its flavor that blends very well with other food ingredients, as well as to its attractive red color.

The food industry produces from the tomato, variety of products that today are popular in practically every kitchen. However, recent findings indicate that in addition to flavor and appearance, the tomato adds to our diet certain phytonutrients that are important to our health and well being.

LycoRed Natural Products Industries decided to separate those phytonutrients and to make them available to the food and nutraceutical industries.

Following a prolonged multi-disciplinary research, LycoRed has developed an integrated industrial process to produce several new tomato ingredients from the tomato. While food industry uses the whole tomato, either peeled, sliced, crushed, or concentrated, the LycoRed process separates the fruit into three fractions. Each one has special functional properties that open new possibilities to the food manufacturer:

I. Tomato oleoresin

This fraction consists of tomato lipids and contains high concentration of Lycopene and other important phytonutrients. The oleoresin is the basis for the production of Lyc-O-Mato® range of formulations used in dietary supplements and in functional foods.

II. Soluble tomato solids concentrate

This fraction consists of soluble tomato solids and is available as a concentrate (CTC 60°Bx), or as the dehydrated version (Tomat-O-Pure), which is preferred for certain applications.

Both products are used as a flavor enhancer and can effectively replace MSG in food formulations. Thus, the new tomato based ingredients contribute to the clean label policy.

Soluble tomato solids blend very well in various food formulations such as tomato products, beverages, savory dishes and in fruit bases.

III. Insoluble tomato solids

The third fraction contains the insoluble solids obtained from the red-ripe tomato fruit. There are several products in this category:

One) Lycopene Rich Tomato Pulp (LRTP) – frozen in lined metal drums.

Two) LycoFibers – dehydrated LRTP, contains 1.3% of Lycopene.

Both the frozen and dehydrated pulp products contain Lycopene in its native state, still attached to tomato tissue. They are used as a source of Lycopene in functional foods.

c) NFT tomato fibers – NFT tomato fibers consists of the dehydrated tomato pulp after solvent extraction of the oleoresin. These dietary fibers have unique water absorption capacity (1+16) and are used to regulate viscosity, improve texture and to prevent syneresis in food products.

THE EFFECT OF NATURAL LYCOPENE, LYC-O-MATO® AND SYNTHETIC LYCOPENE ON EXERCISE-INDUCED ASTHMA. Ben-Amotz, A., Neuman, I. The National Institute of Oceanography, Haifa, and Allergy Department, Rabin Medical Center, Petach Tikva, Israel

Lycopene was previously shown to have high antioxidative activity. In attempt to clarify the controversy regarding the beneficial effect of antioxidants on asthma, the acute effects of natural lycopene, LYC-O-MATO®, tomato extract, a product of Lycored Natural Products Industries Ltd., and synthetic lycopene were assessed on airway hyper-reactivity in patients with exercise-induced asthma (EIA).

Thirty patients with EIA participated in our study to verify the antioxidative effect. The test was based on the following sequence: baseline pulmonary function, 7 minutes exercise session on a motorized treadmill, 8 minutes rest and again pulmonary function. One week oral random, double-blind supplementation of placebo, 30 mg/day LYC-O-MATO® (20 patients) or 30 mg/day synthetic lycopene (10 patients), pulmonary functions at rest, 7 minutes exercise session, 8 minutes rest and again pulmonary functions. Four weeks washout interval was applied between the placebo to the lycopene supplementation.

All patients given placebo showed a significant post exercise reduction of more than 15% in their forced expiratory volume in one second (FEV_1). Of the same patients after receiving a daily dose of 30 mg of lycopene in LYC-O-MATO® capsules for 1 week, the patients were significantly protected against EIA. Patients receiving 30 mg synthetic lycopene per day for 1 week were not protected against EIA. Serum analyses of the patients by high-pressure liquid chromatography detected in the LYC-O-MATO® and in the synthetic lycopene supplemented patients elevated level of lycopene compared to the placebo with no change in retinol, tocopherols or in the other carotenoids. Lower doses of 15 mg per day natural lycopene were not effective.

Our results indicate that a daily dose of 30 mg lycopene in capsules of LYC-O-MATO® exerts a protective effect against EIA in asthmatic patients. Comparative study with synthetic lycopene was not significantly effective. The results can be explained most probably through in vivo lipophilic antioxidative synergistic effect of lycopene and other(s) tomato extracted ingredient.

LYCOPENE AND ASTAXANTHIN INHIBIT PROLIFERATION OF HUMAN PROSTATE CANCER CELLS INDUCED BY ANDROGENS AND IGF-I.

Nir Z*, Agemy L, Danilenko M, Kirilov E, Giat Y, Sharoni Y and Levy J.
*LycoRed, Natural Products Industries Ltd. Beer-Sheva, and Department of Clinical Biochemistry, Faculty of Health Sciences, Ben-Gurion University and Soroka Medical Center, Beer-Sheva, Israel.

The role of androgens and estrogens in prostate and breast cancer is well established. Recently, it has been found that insulin-like growth factor-I (IGF-I) blood levels are a powerful risk factor for prostate cancer (similar to the way that cholesterol levels predict the risk of cardiovascular diseases). The same group of investigators also reported that intake of the tomato carotenoid lycopene reduces the risk of prostate cancer.

Our purpose was to find whether carotenoids inhibit prostate cancer cell growth and to suggest a molecular mechanism for their anticancer activity in human prostate cancer.

We used [^{3}H]thymidine incorporation and cell counting to measure the proliferation of androgen-dependent (LNCaP) and-independent (DU-145) human prostate cancer cells.

Dihydrotestosterone (DHT) stimulated cell growth and increased secretion of Prostatic Specific Antigen (PSA) in LNCaP cells but did not affect DU-145 cells. Lycopene and astaxanthin already inhibited the androgen-induced stimulation on the first day of incubation. The IC_{50} for lycopene was about 1 μM, which is well within the range of the carotenoid level in human plasma. Low levels of lycopene (0.3 μM) did not significantly affect MCF-7 mammary cancer cell growth. Combination of the two carotenoids (lycopene and astaxanthin) synergistically inhibited cell proliferation at the low close to physiological levels. IGF-I was found to be a strong mitogen for both types of cells. Lycopene and astaxanthin inhibited basal and IGF-I stimulated growth. This inhibition was accompanied by slow down of cell cycle progression from G1 to S phase. Analysis of molecular components of the cell cycle machinery, which can explain these effects, will be presented.

This report provides an explanation for the anticancer activity of a carotenoid rich diet that has been recently suggested in epidemiological studies. The mechanism of this protection is related to reduction in the activity of androgens and IGF-I important risk factors for prostate cancer.

BIOAVAILABILITY OF LYCOPENE, THE RED TOMATO CAROTENOID, IN SERUM AND TISSUES OF PATIENTS UNDERGOING ELECTIVE AND CANCER SURGERY

Nir Z*, Walfisch S, Walfisch Y, Ermoshkin A, Mnitentag H., Agbaria R. Levy J. and Sharoni Y., *LycoRed, Natural Products Industries Ltd. Beer-Sheva, Departments of Clinical Biochemistry, Pharmacology and the Colorectal Unit, Faculty of Health Sciences, Ben-Gurion University of the Negev and Soroka Medical Center, Beer-Sheva, Israel

Carotenoids, like many other micronutrients, are not synthesized in the human body and thus their tissue and serum levels reflect the amounts consumed from the diet. However, its is not known if supplementation can affect normal and malignant target tissue levels of lycopene. Insulin-like growth factor-I (IGF-I) blood level is a powerful risk factor for prostate and breast cancer. Dietary means are superior preventive strategies for the long term reduction of such risk.

Our aim focused on two main topics: First to determine the relationships between concentrations of lycopene in human plasma and body tissues after lycopene supplementation. Second to find if lycopene supplementation will reduce IGF-I blood levels.

We measured the absorption of dietary lycopene in two groups of patients a) those undergoing elective hemorrhoidectomy and b) those undergoing surgery due to colon and breast cancer. Tomato lycopene oleoresin (twice 15 mg/day) or a placebo were administrated for several days (cancer patients) or three to four weeks (elective surgery patients) prior to surgery. Lycopene concentration and isomer distribution in blood and in the surgically removed tissues was measured by HPLC. Several components of the IGF system were also measured in bloods of these patients by routine methods.

In hemorrhoidectomy patients the plasma of lycopene increased after supplementation from 0.31±0.021 nmol/ml to 0.56±0.053. In the placebo group the lycopene plasma level did not change significantly. The increase in both skin and adipose tissue lycopene levels in the lycopene-treated group as compared to the placebo group was 1.4 fold. Lycopene supplementation did not significantly change the proportion of *all-trans* v.*cis* isomer in the plasma and tissues, despite the fact that more than 90% of the supplemented lycopene was in the *all-trans* form. The lack of change in the isomer distribution after supplementation suggests that the *cis*-isomers are preferentially absorbed or that the *all-trans*-lycopene is isomerized to the *cis*-form after absorption. In the cancer patients the increase in plasma lycopene level was lower than in the hemorrhoidectomy patients probably because of the shorter supplementation period. However, a significant increase in the tumor tissue content was evident in the lycopene-treated group (0.72±0.15) as compared to the placebo group (0.54±0.09 nmol/gr).

In 15 of the 28 patients in the lycopene-treated group, IGF-I blood levels decreased more than 10% after supplementation. In the placebo-treated group, there was no change in IGF-I levels in 20 of the 28 patients.

These results show that lycopene supplementation increases lycopene levels in serum and in target tissues. The preliminary, promising, results on IGF-I plasma levels should be extended and confirmed in future studies. The ability to increase lycopene levels in target tissues is a prerequisite for using the carotenoid as a food supplement that may prevent or affect tumor progression.

MODULATION OF EXPERIMENTAL COLON CARCINOGENESIS BY NATURALLY-OCCURRING β-CAROTENE, LUTEIN AND β-IONONE. Cooma, I., Swamy, M.V., Simi, B., Steele, V.E., Reddy, B.S., and Rao, C.V. Chemoprevention Program, American Health Foundation, Valhalla, NY, and Division of Cancer Prevention and Control, National Cancer Institute, Bethesda, MD.

Epidemiological studies suggest that naturally-occurring antioxidants such as β-carotene and related compounds play an important role in reducing risk of several cancers; although, recent clinical studies suggest that β-carotene may cause the enhancement of certain type of cancers. We have assessed the chemopreventive efficacy of β-carotene, lutein and β-ionone (a metabolic intermediate of carotenoid biosynthesis, structurally similar to retinoic acid) on azoxymethane (AOM)-induced colon carcinogenesis, using aberrant crypt foci (ACF) as a marker. Prior to the efficacy study, we evaluated the maximum tolerated dose (MTD) of dietary β-carotene, lutein and β-ionone in male F344 rats by administering different concentrations (0-4000 ppm) each agent in a 6-week subchronic toxicity study. Based on body weights reduction and external signs of toxicity, the MTD of β-carotene, lutein and β-ionone was found to be 2,500 ppm for each agent in male F344 rats. In the bioassay, β-carotene and lutein were tested either at low dose levels (4 and 8% MTD) or high dose (40 and 80% MTD) levels; β-ionone was tested at 40 and 80% MTD levels. Five week-old male F344 rats were fed the control diet (modified AIN-76A) or experimental diets containing 100 and 200 ppm or 1,000 and 2,000 ppm of β-carotene and lutein or 1,000 and 2,000 ppm of β-ionone. Two weeks later, all animals except those in the vehicle (normal saline)-treated groups were s.c. injected with AOM (15 mg/kg body wt., once weekly for 2 weeks). At 14 weeks of age, all rats were killed, and their colons evaluated for ACF. Administration of the 4 and 8% MTD level of β-carotene significantly inhibited AOM-induced total colonic ACF formation by 24% and 36%, respectively; whereas lutein produced inhibition only at the 8% MTD level (27%; $P<0.02$) but had no significant effect at the 4% MTD level. Interestingly, administration of the high level (40 and 80% MTD) doses of β-carotene (124% and 144%) and lutein (110% and 159%) increased colonic ACF formation in a dose-dependent manner ($P<0.04$-0.004). In contrast, β-ionone at 40 and 80% MTD levels significantly suppressed colonic ACF. These results suggest that chemopreventive activity β-carotene, lutein and β-ionone against colon carcinogenesis depends on the dose levels applied. Further studies are warranted to understand the pro- and anti-carcinogenic activities of these naturally-occurring antioxidants.

LYCOPENE CONCENTRATIONS IN PLASMA AFTER CONSUMPTION OF TABLETS CONTAINING LYCOPENE IN BEADLETS OR LYCOPENE FROM NATURAL SOURCES. [1]Cohn, W., [1]Schalch, W., [2]Thürmann, P., [1]Aebischer, C., [1]Schierle, J., [3]Elliott, J. F. Hoffmann-La Roche LTD[1], Basel, Switzerland; University of Wuppertal[2], Wuppertal, Germany; Roche Vitamins Inc.[3], Parsippany, New Jersey, USA.

Carotenoid bioavailability from natural sources is determined by several factors. In particular, the release of carotenoids from the cell-matrix in which they are incorporated impacts intestinal absorption. Accordingly, disruption of the plant cell walls and carotenoid complexes by measures such as cooking and grinding improves β-carotene absorption. Similarly, lycopene bioavailability was demonstrated to depend on tomato processing, as bioavailability from tomato paste exceeded that from raw tomato and its juice. The relative bioavailability of lycopene from water-dispersible formulations (beadlet formulations) of lycopene and tomato food products has not yet been determined.

The purposes of this study were: (1) to compare the lycopene concentrations in plasma during and after regular consumption of tablets containing synthetic lycopene in beadlets or lycopene from natural sources in the form of tomato juice or tomato soup, (2) to provide plasma data on major lycopene isomers, and (3) to estimate lycopene plasma half-life for these carotenoid forms.

In a randomized, parallel group study design, volunteers (N= 6 per group) were advised to minimize their dietary lycopene intake for 2 weeks before they were dosed with approximately 20 mg of lycopene over 7 days from tomato juice, tomato soup or tablets (Lycopene 5% TG, Roche). Thereafter, lycopene post-dosing kinetics were monitored for an additional three weeks. Plasma lycopene was analyzed with normal-phase HPLC. The all-trans and cis isomers of lycopene were detected and quantified at a wavelength of 470 nm. Over the time course of the study, volunteers were required to maintain the dietary restrictions. Irrespective of the lycopene treatment, the all-trans form was the predominant lycopene isomer whereas 5-cis lycopene was the most abundant cis form. For total and all-trans lycopene a comparable plasma response was observed for intake of lycopene from tomato soup and tablets. By contrast, availability from tomato juice was significantly lower ($p<0.05$). Relatively high 5-cis lycopene plasma concentrations resulted from lycopene administration in tablets, as the isomer was most abundant in this formulation. However, these differences disappeared after dose normalization of the plasma concentrations. The variability of lycopene concentrations could be reduced by normalization to plasma cholesterol levels. Half-life was similar for lycopene administered in tomato soup or tablets and was of the order of 5 to 7 days.

The study confirms the importance of food processing for improving carotenoid availability. Appropriate food processing resulted in systemic lycopene availability comparable to that of synthetic lycopene formulated in tablets.

FXR IS ACTIVATED BY HERBS AND SPICES IN TOMATO-BASED FOOD PRODUCTS: IMPLICATIONS FOR CHOLESTEROL AND GROWTH CONTROL. Weinberger, C., Burns, E., and Goode, E. National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Our studies are directed at identifying effector molecules for FXR, a member of the nuclear receptor superfamily. An FXR-dependent ligand-responsive assay was assembled by cotransfecting CHO cells with an FXR-expressing plasmid DNA and a plasmid consisting of an FXR-responsive DNA element placed upstream of the bacterial chloramphenicol acetyltransferase (CAT) reporter gene. Increases in CAT activity from lysates of cells treated with candidate chemicals were then measured against lysates from parallel vehicle-treated cells to define FXR effectors. We and others have shown that FXR activates transcription in response to farnesol and its metabolites and bile acids. Recently we uncovered many other FXR-activating isoprenoid metabolites. These mevalonate-derived products include 20R- and 22R-hydroxycholesterol, the 3α-hydroxylated metabolites of all classes of adrenal steroids (glucocorticoids, progestins, and androgens), oxylanosterols, and ubiquinones with short isoprenylated side chains. Its activation by this isoprenoid metabolic flux ideally positions FXR as a feedback regulator of HMG CoA reductase activity, which is responsible for synthesizing mevalonate, the limiting biosynthetic precursor to cholesterol and all cellular isoprenoids. Mevalonate is essential for the growth of all cells. FXR is thus ideally positioned as a coordinate feedback regulator of isoprenoid synthesis and cell growth. In addition to these endogenously-produced isoprenoids, FXR-dependent transcription was induced by juvenile hormone III, an endogenously-produced terpenoid produced by insects. Juvenile hormones exhibit insecticidal actions when applied exogenously to insect larvae undergoing metamorphosis. We now show that FXR was activated by synthetic juvenile hormones in addition to diverse classes of man-made insecticides including pyrethrins, organophosphates, organochlorines, and the insecticide synergist piperonyl butoxide. In addition to these synthetic insecticides, FXR-dependent activity was modulated by structurally-diverse secondary metabolites that are synthesized by plants to help defend against insect herbivory. These compounds include ones from herbs and spices such as savory, thyme, oregano, basil, garlic, and olive oil which constitute the so-called "Italian seasonings" that are typically added to most tomato-based food products. It has been shown that these FXR-activating dietary ingredients from plants may either modulate plasma cholesterol levels or exert carcinogenic or anti-carcinogenic effects in animals. Through its ability to modulate the production of growth-requiring isoprenoids, FXR may thus help to integrate the mitogenic effects of dietary carcinogens and their anti-carcinogenic metabolites that are found not only in ingredients associated with tomato-based food products, but in many other phytochemicals in human and animal diets.