Research Abstracts: Green Tea

VEGF receptor phosphorylation status and apoptosis is modulated by a green tea component, epigallocatechin-3-gallate (EGCG), in B-cell chronic lymphocytic leukemia.

We recently reported that chronic lymphocytic leukemia (CLL) cells synthesize and release vascular endothelial growth factor (VEGF) under normoxic and hypoxic conditions. CLL B cells also express VEGF membrane receptors (VEGF-R1 and VEGF-R2), suggesting that they use VEGF as a survival factor. To assess the mechanism of apoptosis resistance related to VEGF, we determined the impact of VEGF on CLL B cells, and we studied the impact of epigallocatechin-3-gallate (EGCG), a known receptor tyrosine kinase (RTK) inhibitor, on VEGF receptor status and viability of CLL B cells. VEGF165 significantly increased apoptotic resistance of CLL B cells, and immunoblotting revealed that VEGF-R1 and VEGF-R2 are spontaneously phosphorylated on CLL B cells. EGCG significantly increased apoptosis/cell death in 8 of 10 CLL samples measured by annexin V/propidium iodide (PI) staining. The increase in annexin V/PI staining was accompanied by caspase-3 activation and poly-adenosine diphosphate ribose polymerase (PARP) cleavage at low concentrations of EGCG (3 microg/mL). Moreover, EGCG suppressed the proteins B-cell leukemia/lymphoma-2 protein (Bcl-2), X-linked inhibitor of apoptosis protein (XIAP), and myeloid cell leukemia-1 (Mcl-1) in CLL B cells. Finally, EGCG (3-25 microg/mL) suppressed VEGF-R1 and VEGF-R2 phosphorylation, albeit incompletely. Thus, these results suggest that VEGF signaling regulates survival signals in CLL cells and that interruption of this autocrine pathway results in caspase activation and subsequent leukemic cell death. Lee YK, Bone ND, Strege AK, et al. *Blood*. 2004 Aug 1;104(3):788-94.

Induction of apoptosis in prostate cancer cell lines by the green tea component (-)epigallocatechin-3-gallate

Green tea components exert many biological effects, including antitumor and cancer preventive activities. In the search for anticancer agents for prostate cancer the inhibitory effects of green tea components were tested on the prostate cancer cell lines LNCaP, PC-3 and DU145. (-)-Epigallocatechin-3-gallate (EGCG) proved to be the most potent catechin at inhibiting cell growth. The inhibition induced by EGCG was found to occur via apoptotic cell death as shown by changes in nuclear morphology and DNA fragmentation. Thus, we report the first evidence that EGCG is the active component in green tea and induces apoptosis in human prostate cancer cells.Paschka AG, Butler R, Young CY. *Cancer Lett* 1998 Aug 14;130(1-2):1-7.

Green tea and cancer chemoprevention

Worldwide interest in green tea as a cancer preventive agent for humans has increased, because it is non-toxic and it is effective in a wide range of organs. (-)-Epigallocatechin gallate (EGCG) is the main constituent of green tea; the others are (-)-epicatechin gallate, (-)-epigallocatechin and (-)-epicatechin (EC). This paper reports the results of our latest pharmacological and biochemical studies with 3H-EGCG, along with studies on human subjects. The study on bioavailability of 3H-EGCG in Specifically, radioactivity was found in all reported target organs of EGCG and green tea extract (digestive tract, liver, lung, pancreas, mammary gland and skin) as well as other organs (brain, kidney, uterus and ovary or testes) in mice. Recently, we demonstrated that EC enhanced incorporation of 3H-EGCG into human lung cancer cell line PC-9 cells. EC along with another cancer preventive agent sulindac also synergistically enhanced apoptosis in PC-9 cells induced by EGCG. Moreover, a case-control study on breast cancer patients revealed that high daily consumption of green tea was associated

with a lower recurrence rate among Stages I and II patients. All the results suggest that consumption of green tea is a practical and effective cancer preventive both before cancer onset and after cancer treatment. Suganuma M, et al. *Mutat Res* 1999 Jul 16;428(1-2):339-44.

Inhibition of mammary gland carcinogenesis by green tea catechins and other naturally occurring antioxidants in female Sprague-Dawley rats pretreated with 7,12-dimethylbenz[alpha]anthracene

Effects of the naturally occurring antioxidants on mammary gland carcinogenesis were examined in female Sprague-Dawley rats pretreated with 7,12-dimethylbenz[alpha]anthracene (DMBA). Groups of 15-16 7-week-old rats received a 50 mg/kg body weight intra-gastric dose of DMBA, and starting one week thereafter placed on diet containing 0.4% catechol, 1.0% gamma-oryzanol, 2.0% phytic acid, 1.0% green tea catechins (GTC), 1.0% tannic acid or basal diet alone for 35 weeks. Although the final incidences and multiplicities of mammary tumors were not significantly different between DMBA-treated groups, the numbers of survivors in the antioxidant-treated groups at the end of the experiment at week 36 were significantly higher than in the basal diet group. In particular, the survival rate of the GTC group at 93.8% strongly contrasted with that of only 33.3% for rats on the basal diet. At the end of week 18, when all the animals were still alive, the average size of palpable mammary tumors was significantly smaller in the catechol, phytic acid and catechins groups. These results indicate that antioxidants, and GTC in particular, inhibit rat mammary gland carcinogenesis after DMBA initiation. Hirose M, Hoshiya T, Akagi K, Futakuchi M, Ito N. *Cancer Lett* 1994 Aug 15;83(1-2):149-156.

Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans

BACKGROUND: Current interest in the role of functional foods in weight control has focused on plant ingredients capable of interfering with the sympathoadrenal system. OBJECTIVE: We investigated whether a green tea extract, by virtue of its high content of caffeine and catechin polyphenols, could increase 24-h energy expenditure (EE) and fat oxidation in humans. DESIGN: Twenty-four-hour EE, the respiratory quotient (RQ), and the urinary excretion of nitrogen and catecholamines were measured in a respiratory chamber in 10 healthy men. On 3 separate occasions, subjects were randomly assigned among 3 treatments: green tea extract (50 mg caffeine and 90 mg epigallocatechin gallate), caffeine (50 mg), and placebo, which they ingested at breakfast, lunch, and dinner. RESULTS: Relative to placebo, treatment with the green tea extract resulted in a significant increase in 24-h EE (4%; $P < 0.01$) and a significant decrease in 24-h RQ (from 0.88 to 0.85; $P < 0.001$) without any change in urinary nitrogen. Twenty-four-hour urinary norepinephrine excretion was higher during treatment with the green tea extract than with the placebo (40%, $P < 0.05$). Treatment with caffeine in amounts equivalent to those found in the green tea extract had no effect on EE and RQ nor on urinary nitrogen or catecholamines. CONCLUSIONS: Green tea has thermogenic properties and promotes fat oxidation beyond that explained by its caffeine content per se. The green tea extract may play a role in the control of body composition via sympathetic activation of thermogenesis, fat oxidation, or both. Dulloo AG, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M, Chantre P, Vandermander J. *Am J Clin Nutr* 1999 Dec;70(6):1040-5.

Regular consumption of green tea and the risk of breast cancer recurrence: follow-up study from the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC), Japan

Experimental studies suggest various features of anticancer activity of green tea including inhibitory effect of tumor invasion and metastasis. This study was conducted to examine the association between regular green tea consumption prior to diagnosis and subsequent risk of breast cancer recurrence. The Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC) was started in 1988, in which information on lifestyle has routinely been collected from all first-visit outpatients by questionnaire. A total of 1160 new surgical cases of female invasive breast cancers with HERPACC information diagnosed between June 1990 and August 1998 were followed up through December 1999, and the risk (hazard ratio: HR) of recurrence

was assessed with reference to daily green tea consumption using a Cox proportional hazard model. During 5264 person-years of follow-up, 133 subjects (12%) were documented to suffer recurrence of breast cancer. A decreased HR for recurrence adjusted for stage was observed with consumption of three or more daily cups of green tea (HR=0.69, 95% confidence interval (95%CI)=0.47-1.00). Particularly in stage I, the HR was decreased statistically significantly (HR=0.43, 95%CI=0.22-0.84). A similar tendency was observed for stage II subjects, but was not present among more advanced stages. Although careful interpretation is needed, these results suggest the possibility that regular green tea consumption may be preventive against recurrence of breast cancer in early stage cases. Inoue M et al. *Cancer Lett* 2001 Jun 26;167(2):175-82.

Growth inhibition, cell-cycle dysregulation, and induction of apoptosis by green tea constituent (-)-epigallocatechin-3-gallate in androgen-sensitive and androgen-insensitive human prostate carcinoma cells

Prostate cancer (PCA) is the most prevalent cancer diagnosed and the second leading cause of cancer-related deaths among men in the United States. Descriptive epidemiological data suggest that androgens and environmental exposures play a key role in prostatic carcinogenesis. Since androgen action is intimately associated with proliferation and differentiation, at the time of clinical diagnosis in humans most PCA represent themselves as a mixture of androgen-sensitive and androgen-insensitive cells. Androgen-sensitive cells undergo rapid apoptosis upon androgen withdrawal. On the other hand, the androgen-insensitive cells do not undergo apoptosis upon androgen blocking, but maintain the molecular machinery of apoptosis. Thus, agents capable of inhibiting growth and/or inducing apoptosis in both androgen-sensitive and androgen-insensitive cells will be useful for the management of PCA. In the present study, we show that (-)-epigallocatechin-3-gallate (EGCG), the major polyphenolic constituent present in green tea, imparts antiproliferative effects against both androgen-sensitive and androgen-insensitive human PCA cells, and this effect is mediated by deregulation in cell cycle and induction of apoptosis. EGCG treatment was found to result in a dose-dependent inhibition of cell growth in both androgen-insensitive DU145 and androgen-sensitive LNCaP cells. In both the cell types, EGCG treatment also resulted in a dose-dependent G(0)/G(1)-phase arrest of the cell cycle as observed by DNA cell-cycle analysis. As evident by DNA ladder assay, confocal microscopy, and flow cytometry, the treatment of both DU145 and LNCaP cells with EGCG resulted in a dose-dependent apoptosis. Western blot analysis revealed that EGCG treatment resulted in (i) a dose-dependent increase of p53 in LNCaP cells (carrying wild-type p53), but not in DU145 cells (carrying mutant p53), and (ii) induction of cyclin kinase inhibitor WAF1/p21 in both cell types. These results suggest that EGCG negatively modulates PCA cell growth, by affecting mitogenesis as well as inducing apoptosis, in cell-type-specific manner which may be mediated by WAF1/p21-caused G(0)/G(1)-phase cell-cycle arrest, irrespective of the androgen association or p53 status of the cells. Gupta S, Ahmad N, Nieminen AL, Mukhtar H. *Toxicol Appl Pharmacol* 2000 Apr 1;164(1):82-90.