

Nutrition Congress 2015: Enhanced chemo-preventive effect by combining quercetin and green tea in prostate cancer- Piwen Wang- Charles R Drew University of Medicine and Science

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Abstract

The chemo-preventive action of green tea (GT) green tea polyphenols (GTPs) in prostate cancer has is demonstrated well in preclinical cell culture and animal models. However, results from human studies are inconsistent. The low bioavailability and extensive metabolism of GT polyphenols (GTPs) in vivo bound the anti-cancer activity of GT. We determined whether a methylation inhibitor quercetin (Q) will enhance the chemoprevention of prostatic adenocarcinoma in vivo. We found in human prostate tissues and in mouse xenograft prostate tumor tissues that around 50% of GTPs were in methylated form after GT consumption and therefore the methylation decreased the anticancer activity of GTPs. We determine that the mix of a natural methylation inhibitor quercetin (Q) with GT increases the cellular concentrations of GTPs 4-10 fold in prostatic adenocarcinoma LNCaP and PC-3 cells and decreases the methylation of GTPs. This mixture treatment enhanced the inhibition of cell proliferation and induction of apoptosis in both cell lines. Then we performed an animal study to verify the combined effect of GT and Q in vivo. Severe combined immune deficient (SCID) mice were implanted with androgen-sensitive LAPC4 prostatic adenocarcinoma cells and treated with GT, Q, GT+Q or control. Androgen-sensitive LAPC4 prostatic adenocarcinoma cells were injected subcutaneously into severe combined immunodeficiency (SCID) mice one week before the intervention. The concentration of GTPs in brewed tea administered as beverage was 0.07% and Q was supplemented in diet at 0.2% or 0.4%. After 6-weeks intervention the tumor growth was inhibited by 16% (Q), 21% (GT), and 45% (GT+Q) compared to regulate. The tissue concentrations of non-methylated GTPs were significantly increased in the combination group, and were associated with a decreased protein expression of catechol-O-methyl transfer and multidrug resistance-associated protein (MRP)-1. The combination treatment was also associated with a significant increase in the inhibition of proliferation, androgen receptor (AR) and phosphatidylinositol 3-kinases (PI3K)/Akt signaling, and stimulation of apoptosis. The combined effect of GT+0.4% Q on tumor inhibition was further confirmed in another experiment where the intervention started before

tumor inoculation. The combination enhanced the inhibition of protein expression of androgen receptor, prostate-specific antigen and vascular endothelial growth factor.

Quercetin (Q) is a flavonoid that is found in most vegetables and fruits that are edible, particularly in onions, apples, and red wine. The inhibitory effect of Q on the actions of MRPs and COMT has been documented well. Q itself has been shown to exhibit chemo preventive activities specially in prostate cancer. We were able to demonstrate in vitro that the combined use of Q with GT significantly increased the cellular concentrations of non-methylated EGCG in prostate cancer LNCaP and PC-3 cells, leading to enhanced anti-proliferative effects. The present study was designed to test the hypothesis that the combined effect of Q and GT in vivo leads to an increased anticarcinogenic effect in a xenograft prostate tumor mouse model using severe combined immune deficiency (SCID) mice and to elucidate the mechanisms of the increased anticarcinogenic effect of the combination treatment.

The effect of the combination treatment was related to the concentration of GTPs in tumor tissue, which in turn was dependent on the Q dose. The dose of GT used in this study is equivalent to the consumption of 5-6 cups of green tea per day for an adult human. This estimate is based on the observation that the consumption of 5-6 cups of tea daily achieved similar tissue concentrations in human prostate compared to tissue in mice consuming the same brewed GT. Q dose would be equivalent to 1.0g (low dose) and 2.0g (high dose) per day for an adult based on blood concentrations of Q and its metabolites as observed in the present study (data not shown) relative to that from a human study. The consumption of 1000 mg of Q per day was not associated with any adverse effects in humans. A pilot clinical trial is on-going to determine the Q concentration necessary in humans to increase the bioavailability of EGCG. Our results showed that the combination treatment decreased the protein expression of MRP1 in tumor tissues. However, no changes of mRNA expression of MRP1 were observed, indicating that post-transcriptional regulation such as microRNA (miRNA) may be responsible. Many polyphenols including GT and Q have been shown to modulate the expression of miRNA, a class of small

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non-coding RNAs that interact with mRNA to regulate the gene expression post-transcriptionally. Several other investigators demonstrated the inhibitory effects of Q on the activities of transport-regulating proteins such as p-glycoprotein and MRPs, leading to an increased absorption of GTPs from the intestinal tract and retention in the tissues. Although Q is extensively methylated, sulfated, or glucuronidated upon uptake it has been demonstrated that these Q metabolites, such as isorhamnetin and 7-O-glucuronosyl quercetin exhibited equal or stronger inhibition on the activities of MRPs compared to Q. Considering the importance of MRPs in the development of chemo resistance during chemotherapy, GT and Q may also be good candidates to be combined with chemotherapy drugs to reduce drug resistance and enhance therapeutic efficacy.

The important role of catechol O-methylation of GTPs in cancer prevention has been demonstrated in several studies. Due to a common polymorphism of COMT its activity can vary by 3 to 4-fold. A case control study in Asian-American women provided evidence that the risk of breast cancer was significantly reduced only among tea drinkers possessing at least one low-activity COMT allele. We found earlier that EGCG was extensively methylated in human prostate tissues obtained from prostatectomy and in mouse tissues after GT consumption. In cell culture experiments methylation significantly decreased the anticancer activities of EGCG as shown by our laboratory and other investigators. Previously we demonstrated *in vitro* that the combination of GT and Q significantly decreased the activity and protein expression of COMT in various cancer cell lines. This inhibition of COMT was associated with a decrease in EGCG methylation and increase in the anti-proliferative activity. Similarly, Landis-Piowar et al. demonstrated that EGCG treatment in breast cancer cells of lower COMT activity led to stronger proteasome inhibition and apoptosis induction. The present study confirmed the inhibition of COMT *in vivo* both in mRNA and protein expression by the combination treatment of GT and Q, which may contribute to the increased concentrations of non-methylated EGCG in tumor tissues and supports the important role of COMT in GT chemoprevention.

Cancer results from a multistage process with distinct molecular and cellular alterations. Therefore, treatments targeting many concerted processes may be advantageous in cancer prevention, therapy and reducing resistance to the treatment. Natural compounds such as GT and Q target multiple events and signaling pathways throughout the stages of carcinogenesis. In combination these compounds may increase the anticarcinogenic activity by expanding the coverage of molecular targets. The androgen receptor (AR) signaling pathway plays a critical role in prostate tumor growth and progression, thus it is an important target in prostate

cancer prevention and treatment. Nevertheless, there are other signaling pathways particularly the phosphatidylinositol 3-kinases/Akt/ mammalian target of rapamycin (PI3K/Akt/mTOR) pathway that crosstalk with AR signaling and may directly regulate the expression and activation of AR. The upregulation and activation of PI3K/Akt/mTOR pathway is thought to play an important role in prostate cancer due to the decreased expression or loss of the negative regulator, tumor suppressor phosphatase and tensin homolog (PTEN). Akt is activated after phosphorylation by phosphorylated PI3K and in turn activates its substrates, one being mTOR, which leads to increased cell proliferation and survival. Therefore, an effective intervention strategy in prostate cancer may need to target both AR and PI3K/Akt/mTOR signaling pathways. Both GT and Q inhibit AR signaling through multiple mechanisms including the decrease of AR expression and its nuclear translocation. The combined use of GT and Q in the present study demonstrated an increasing ability to inhibit AR expression compared to individual treatments. In addition, the phosphorylation of Akt was significantly inhibited by the combination treatment while only a slight but non-significant decrease by the individual treatments. Further evidence of a stronger inhibition of AR and PI3K/Akt signaling was also provided through increased inhibition of AR-mediated PSA expression in tumor tissues from mice treated with GT+Q. Similar effects were demonstrated by a recent study that combined mTOR inhibition (everolimus) with an anti-androgen (bicalutamide) to block both pathways, resulting in tumor growth was statistically significantly reduced. In addition to their applications in cancer prevention, GT and Q may be ideal candidates to be combined with anti-androgens to enhance the therapeutic efficacy in a less-toxic manner in the treatment of advanced prostate cancer.

This study provides a novel regimen by combining GT and Q to enhance the chemoprevention of prostate cancer in a non-toxic manner. This was associated with an increased bioavailability of non-methylated GTPs and enhanced anti proliferative and proapoptotic effect. These results warrant future human intervention studies to confirm the combined effect of GT and Q in prostate cancer prevention and treatment.

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