Why drinking green tea could prevent cancer

Epidemiological studies suggest that the consumption of green tea may help prevent cancers in humans; also, breast and prostate cancers in animal models are reduced by green, but not black, tea[1]. Here we offer a possible explanation. We have inferred (using molecular modelling) and subsequently demonstrated that one of the major ingredients of green tea inhibits urokinase, an enzyme crucial for cancer growth.

Tea is drunk in three forms: black (78%), green (20%) and oolung (2%). Green tea contains many polyphenols known as cathechins, including epigallocathechin-3 gallate (EGCG), epigallocathechin (EGC) and epicathechin-3 gallate (ECG). The brewing of black tea oxidizes the cathechins, destroying any beneficial effects[1]. Several mechanisms of anticancer activity of cathechins have been postulated, but none seems universal for all cancers[1–3].

Human cancers need proteolytic enzymes to invade cells and form metastases. One of these enzymes is urokinase (uPA). Inhibition of uPA can decrease tumour size or even cause complete remission of cancers in mice[4,5]. The known uPA inhibitors are unlikely to be used in anticancer therapy because of their weak inhibitory activity or high toxicity.

We have searched for new uPA inhibitors by computer modelling using the active site of uPA as a template. Coordinates of human uPA were kindly provided by C. Phillips[6]; National Cancer Institute, MayBridge, and Merck 3D databases of 190,000 compounds were used to select inhibitors by Biosym LUDI and DOCKING programs. In these calculations, the relative position of inhibitor and receptor (uPA) with the minimum potential energy represents the most probable way of binding. Polyphenols, among other compounds, showed good inhibitory potential. One of them, EGCG (a component of green tea), binds to uPA, blocking His 57 and Ser 195 of the uPA catalytic triad and extending towards Arg 35 from a positively charged loop of uPA (Fig. 1a). Such localization of EGCG would interfere with the ability of uPA to recognize its substrates and inhibit enzyme activity[6].

We have verified our computer calculations using an amidolytic assay of uPA activity in the presence of different concentrations of EGCG. The uPA activity was quantified spectroscopically using Spectrozyme, which releases a chromogen on specific cleavage by uPA. EGCG from two different suppliers showed almost identical rates of uPA inhibition.

We have compared the ability of EGCG to inhibit uPA with that of a well-known inhibitor, amiloride, and a control sample where no inhibitors were used (Fig. 1b). EGCG is a weaker inhibitor than amiloride, but can be consumed in much higher doses without any toxicological effects. Amiloride is administered in a maximum dose of 20 mg per day, whereas a single cup of tea contains 150 mg EGCG, and some tea lovers consume up to 10 cups a day[1].

Such high levels of a uPA inhibitor are likely to have a physiological effect and could reduce incidence of cancer in humans or the size of cancers already formed. Theoretically, EGCG might inhibit cancer formation in many different ways; however, we postulate that the well-known anticancer activity of green tea is driven by inhibition of uPA, one of the most frequently overexpressed enzymes in human cancers.

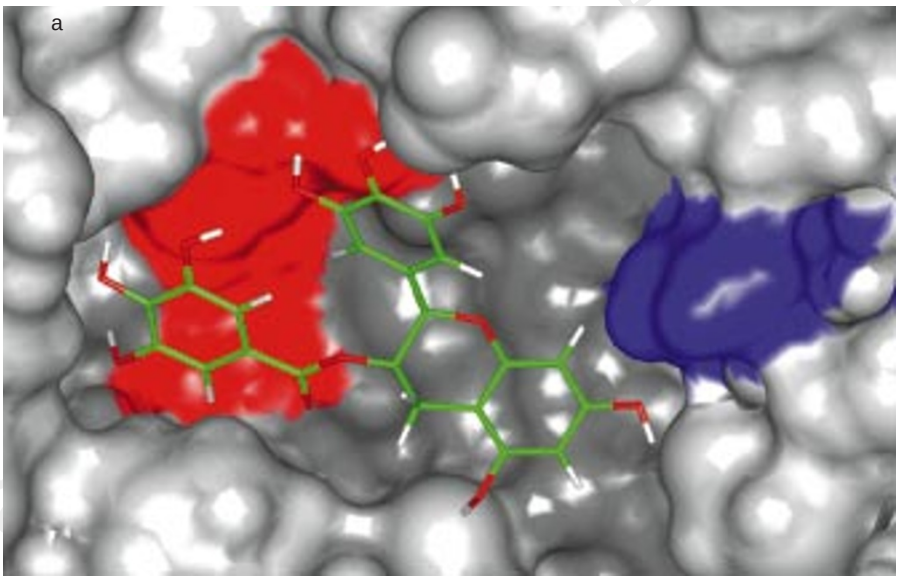

Figure 1a, Connolly surface of uPA showing the catalytic triad His 57, Asp 102 and Ser 195 (red) at the bottom, and Arg 35, Arg A37 (blue) at the brim, of the cavity. EGCG, well fitted into this cavity, is shown as a 'stick model' in green (C), red (O) and white (H). The calculated energy of intermolecular interaction between EGCG and uPA is -116.81 kcal mol^{-1}; LUDI score, 498; calculated K_i, 1.04×10^{-5} M. **b,** Cleavage of Spectrozyme by uPA in the presence of EGCG (inset) from Sigma (blue triangles), and from MayBridge, UK (purple diamonds); amiloride (green squares), and control sample (orange circles). Experimental mixtures (50 mM Tris with 0.01% Tween 80, 0.01% PEG 8000 buffer; pH 8.8) were incubated with 1 μg of uPA and decreasing amounts of inhibitor for 15 min. 100 μl of this mixture was incubated in a 96-well microplate with 50 μl (2.5 mM) Spectrozyme (carbobenzyl-L-(γ)-Glu(α-*t*BuO)-Gly-Arg-*p*-nitroanilide.$2C_2H_5OH$ from American Diagnostica Inc., Greenwich, Connecticut), for 10 min. Absorbance, which is inversely proportional to the uPA inhibitory activity[7], was measured at 405 nm on a microplate reader.

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