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Titel des Beitrags: Activity-guided identification of a chemopreventive compound in coffee beverage using in vitro and in vivo techniques

Abstract:
The aim of the present study was to apply an activity-guided screening procedure to coffee brew to identify a key chemopreventive compound by means of in vitro antioxidant tests as well as cell culture experiments and to prove the in vivo activity of that compound by an animal feeding experiment. Solvent fractionation, followed by multiple-step ultrafiltration, revealed that the polar coffee compounds with molecular weights below 1 kDa show the major inhibitory effect on the in vitro peroxidation of linoleic acid as well as the predominant chemopreventive enzyme modulating activity on the NADPH-cytochrome c reductase (CCR) and glutathione S-transferase (GST) in human intestinal Caco-2 cells. To identify the chemical structure of the most active antioxidants and chemopreventive compounds, the polar compounds were further separated by HPLC techniques, followed by the activity-guided screening of the individual HPLC fraction. These experiments demonstrated 5-chlorogenic acid to be the most powerful antioxidant in vitro, whereas, in contrast, chemopreventive effects on the GST activity were found for the N-methylpyridinium ion, the structure of which was elucidated by LC-MS and NMR experiments and confirmed by synthesis. The in vivo activities of coffee beverage and
N-methylpyridinium ions were tested in a 15-day feeding experiment on rats. In the liver, feeding of 4.5(%) coffee beverage resulted in increases of GST and UDP-GT activities by 24 and 40(%) compared to animals fed the control diet (p > 0.05), respectively. Plasma total antioxidant capacity and plasma tocopherol were elevated in animals fed the coffee beverage and the N-methylpyridinium-containing diet. In summary, the results demonstrating a strong in vitro antioxidant activity for coffee were confirmed by the feeding study. Surprisingly, feeding of N-methylpyridinium also resulted in an increased total antioxidant capacity in the plasma. The data indicate that the mode of action demonstrated for N-methylpyridinium in biological systems is different from that in foods.