Analysis of N^{ε}-ethyllysine in human plasma proteins by NCI-GC/MS as a biomarker for exposure acetaldehyde and alcohol

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Approximately 3.6% of all cancer cases worldwide are related to alcohol consumption. Acetaldehyde, the first metabolite of ethanol, may mediate alcohol-induced carcinogenesis. It is also one of the most prevalent carcinogens in cigarette smoke and is found widely in the environment. N^{ε}-Ethyllysine (NEL) has been reported to be one of the major products formed by the reaction of acetaldehyde with proteins, although its role in carcinogenesis is not fully evaluated due to lack of analytical methods. For these reasons, we have developed a sensitive and specific method to determine NEL in human plasma proteins by negative ion chemical ionization gas chromatography-mass spectrometry (NCI-GC/MS).

Proteins were separated from free amino acids by gel filtration using Sephadex G-15 and hydrolyzed with pronase E. NEL and lysine in the hydrolysates, after adding corresponding stable isotope-labeled internal standards, were derivatized with pentafluorobenzyl (PFB) bromide to form PFB derivatives, which were then quantified by NCI-GC/MS. The detection limit for PFB-NEL was 30 fmol/injection. Using this method, we analyzed NEL in samples of human plasma from 20 healthy volunteers and 112 alcoholic patients. The mean level in alcoholic patients was 1.92 ± 3.22 NEL/10^3 lysines (n=112), which was significantly higher than that of healthy volunteers (0.26 ± 0.06 NEL/10^3 lysines, n=20) ($P < 0.0001$, Welch's *t*-test).

In conclution, we have developed a sensitive and specific method for analyses of NEL in human plasma proteins. The method is applicable to microliters of human plasma. We are currently investigating possible associations between levels of NEL and polymorphisms of the genes encoding for alcohol dehydrogenase (*ADH*) and aldehyde dehyderogenase2 (*ALDH2*).