Analysis of N^{ε}-ethyllysine in human plasma proteins by gas chromatography-negative ion chemical ionization/mass spectrometry as a biomarker for exposure to acetaldehyde and alcohol intake

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Chronic alcohol beverage consumption is a risk factor for cancer of the upper aerodigestive tract, liver, colon, rectum and breast, and thus has been classified as a human carcinogen (group 1) by the International Agency for Research on Cancer. Acetaldehyde, the first metabolite of ethanol, may mediate alcohol-induced carcinogenesis. NEL has been reported to be a major stable adduct formed by the reaction of acetaldehyde with lysine residues in proteins. However, the levels of NEL in tissues have never been reported due to mainly a lack of suitable analytical methods. For these reasons, we have developed a sensitive and specific method to determine NEL in proteins by gas chromatography-negative ion chemical ionization / mass spectrometry (GC-NCI/MS).

This method consists of 1) purification of the protein fraction by Sephadex G-15 to remove low molecular substances, 2) hydrolysis of proteins with Pronase E in the presence of corresponding stable isotope-labeled internal standards, 3) derivatization of amino acids with pentafluorobenzyl (PFB) bromide and 4) analysis of PFB derivatives using a selected ion monitoring mode coupled with GC-NCI/MS.

The developed method was applied to quantitate NEL levels in human plasma proteins. We could detect NEL in plasma proteins obtained from 112 alcoholic patients at 1.94±3.10 NEL / 1000 L-lysine. Pearson's product-moment correlation coefficient revealed that the NEL levels in plasma proteins were statistically-significantly correlated with amounts of alcohol ingested in the previous 24 hours, number of cigarettes smoked per day, alcohol dehydrogenase-1B enzyme activity, average daily amounts of alcohol ingested in the last year and number of times tooth-brushing. Pearson's correlation coefficients (r) were r = 0.21, 0.18, 0.18, -0.18 and -0.24, and the corresponding *P* values for these correlations were *P* = 0.02, 0.04, 0.04, 0.04 and 0.01 respectively. However in stepwise multiple regression analyses, amounts of alcohol ingested in 24 hours, average daily amounts of alcohol ingested in the last year, number of times tooth-brushing and occurrence of cirrhosis were independently correlated with NEL in plasma proteins obtained from alcoholic patients (R^2 = 0.18, *P* <0.001).

The method could be applied to molecular epidemiological studies to investigate possible associations between human diseases and exposure to acetaldehyde and/or alcohol.