DEVELOPMENT OF LABORATORY TESTS FOR ASSESSING VITAMIN STATUS

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ABSTRACT

Aim

To develop assays for the determination of functional vitamin status and to determine the functional vitamin status of an elderly population. Specifically the focus has been to develop assays to measure the biological concentrations of *N,N*-dimethylglycine and glycine betaine as possible markers of functional folate status.

Background

Functional vitamin deficiencies arise when the tissue concentration of a vitamin derived coenzyme is inadequate this can occur despite normal concentrations of the vitamin in the blood or urine, and leads to lower activity of the vitamin dependent enzyme. The lowered enzyme activity causes changes in the concentrations of metabolites associated with the biochemical pathway catalysed by the enzyme. The amount of vitamin at a tissue level can be determined by measuring these changes. Functional vitamin deficiencies have been associated with many chronic diseases; the relationships between nutrients and disease can be investigated using appropriate assays.

Methods and results

Two new trifluromethanesulfonate reagents (2-phenanthrenacyl and 6-methoxynapthacyl) have been synthesised. These reagents form highly fluorescent derivatives with *N,N*-dimethylglycine, glycine betaine and propionylcarnitine. Using 2-phenanthrenacyl triflate as the derivatising reagent the detection limit for glycine betaine is improved from 0.2 μM to 0.04 μM. Optimisation is achieved by changing the solvent, base and water content of the reaction mixture. Polar aprotic solvents are used, with the presence of some water or alcohol tolerated. Suitable bases include the inorganic bases, magnesium hydroxide, silver oxide and lithium phosphate.

The cationic derivatives of *N,N*-dimethylglycine and glycine betaine in plasma are separated by HPLC on an alumina column within 50 minutes. Cation exchange HPLC is carried out using a polar organic solvent containing an aqueous buffer with an organic cation and a hydrophilic anion. Selectivity is affected by the choice of organic solvent and buffer. Increasing the water content and the buffer concentration reduces the retention of the derivatives. Propionylcarnitine can be quantified after separation by HPLC on a

non-endcapped strong cation exchange column however the use of this assay to detect functional biotin deficiency has not been validated.

^{1}H NMR can be used to measure *N,N*-dimethylglycine and glycine betaine in urine. The inter and intra-assay CV's were < 10% and recoveries were ≥ 97% over a linear range from 50 μM to 1000 μM. Limits of detection using ^{1}H NMR spectroscopy (15 – 25 μM) are higher than HPLC assays, though adequate for the detection of raised concentrations in urine.

Elderly hip fracture patients (aged 65-90) were investigated, as they are known to have poor nutrition compared to health elderly, and would be expected to have associated vitamin deficiencies. A greater percentage of hip fracture patients had insufficient vitamin B_{12} and folate concentrations compared to age matched healthy elderly controls (Folate 55% and 32% <120 pM, Vitamin B_{12} 7% and 5% <8.5 nM). The results for other analytes are difficult to interpret because of the affects of recent trauma. In the control population glycine betaine predicts total homocysteine concentrations (multiple linear regression –0.055 P = 0.099) and is a stronger predictor of folate than total homocysteine. The ratio of the concentrations of *N,N*-dimethylglycine/glycine betaine was not significantly associated with folate status.

Conclusion

The concentrations of *N,N*-dimethylglycine and glycine betaine in plasma can be measured by HPLC, and in urine by ^{1}H NMR. In healthy elderly there is a high prevalence of vitamin deficiency. The ratio of *N,N*-dimethylglycine/glycine betaine is not an appropriate marker of functional folate status, however the associations between glycine betaine and homocysteine metabolism require further investigation.

PUBLICATIONS ARISING FROM THIS THESIS

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In closing I would like to thank the anonymous author of my email quote… thank you for keeping it all in perspective!

> "Life should NOT be a journey to the grave with the intention of arriving safely in an attractive and well-preserved body, but rather to skid in sideways, Champagne in one hand, strawberries in the other, body thoroughly used up, totally worn out & screaming 'WOO HOO - What a ride!'"

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LIST OF ABBREVIATIONS

#NOF	fractured neck of femur
^{1}H NMR	proton nuclear magnetic resonance
^{o}C	degrees celsius
CE	capillary electrophoresis
EDTA	*N,N,N,N*-ethylenediamine tetraacetic acid
ELISA	enzyme linked immunosorbent assay
ESI MS/MS	electrospray ionisation tandem mass spectrometry
GC-MS	gas chromatography mass spectrometry
HIV	human immunodeficiency virus
HPLC	high pressure liquid chromatography
hr.	hour
LC-MS/MS	liquid chromatography tandem mass spectrometry
Lihep	lithium heprin
M	$molL^{-1}$
min	minutes
NAD^{+}	nicotinamide adenine dinucleotide
NADH	reduced nicotinaminde adenine dinucleotide
NMR	nuclear magnetic resonance
pK_a	log of the acid dissociation constant
pH	negative log of the hydrogen ion concentration
r	correlation coefficient
RNA	ribonucleic acid
SD	standard deviation
UV	ultra violet
v/v	volume to volume
λ	wavelength