Analysis of Functional Foods: The Fine Line Between Nutritional and Therapeutic Effects

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Introduction

The increasing market appeal of foods that have health benefits beyond the nutritional is defining market strategies for many new food products. Some examples of functional foods include margarine with added cholesterol-lowering phytosterols, breads fortified with fish oil and more recently, palatable soy flour. Justifying claims of functionality is difficult, given the nature of the substances, route of delivery and mechanism of action. In the majority of cases, functional agents are applied in the treatment of chronic conditions or as preventatives against future illness and so measuring efficacy may defy the use of traditional measures.

Analysis of samples requires a multi-disciplinary approach that covers the food from raw materials through to the finished product. As with all therapeutic substances, functional foods are increasingly scrutinised using in vivo studies that explore bioavailability, therapeutic effect, toxicology and metabolism of the active substances. Here, we discuss two functional additives and their metabolic pathways to illustrate the multi-disciplinary requirements to establish a causal chain in the application of the functional approach.

Unsaturated Fish Oils

Fish oils are widely acknowledged to possess anti-inflammatory and anti-oxidant activity but are also known to contribute to neural development of infants. Globally, fish oil is included in mainstream products such as bread, beverages, health supplements and infant formula. While the ω-3 fatty acids are generally acknowledged as the functional agents, uncertainty remains in ascribing the particularly high activity of oil from some species to specific (and unusual) compounds or to bioavailability under normal dietary and extreme conditions.

One species that has reputed activity as an anti-inflammatory agent is the Green-Lipped Mussel (Perna canaliculus). Analysis of the isolated fatty acid fraction (Figure 1) shows that the main oils present are eicosapentanoic acid (C20:5), and docosahexanoic acid (C22:6) (Figure 2). Significant to the elucidation of a functional mechanism, several non-branched fatty acids, the major one being isomeric with arachidonic acid (C20:4), were further separated using a BPX70 column (chromatogram not shown).

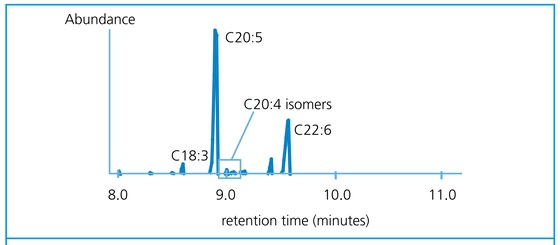

Figure 1. Total Ion Chromatogram of fish oils obtained from Green-Lipped Mussels (Perna canaliculus). Chromatogram shows the FAME fraction containing four and five double bonds, obtained using a BPX5 (15 m x 0.25 mm x 0.25 µm) column.

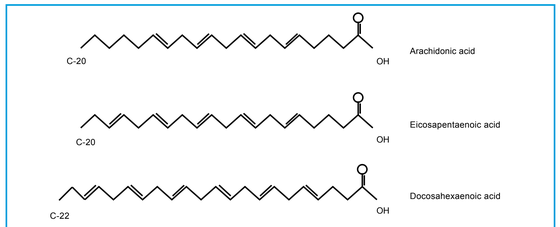

Figure 2. The functional components of fish oil, identified using GC resolution on a BPX70 column.

Determining whether Perna canaliculus possesses a minor fatty acid with 'magic bullet'-like activity or simply has a more bioavailable pool of ω-3 fatty acids required an understanding of the metabolic pathways for these functional components (Figure 3). The selective use of both COX and LTB4 generating assays for various fatty fractions processed from the mussel and isolated pure fatty acids suggests that overall free fatty acid bioavailability and not highly active minor components are responsible for the activity of the species (McPhee et al. 2001, 2003).

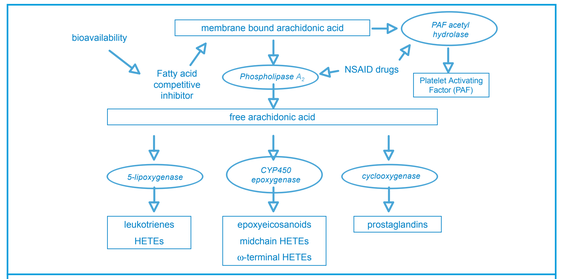

Figure 3. The arachidonic acid cascade and the point at which fish oil can exert its competitive inhibitory effects on the lipooxygenase and cyclooxygenase pathways.

Implications of the Study

The identification of specific compositions of total lipid as contributing to the activity of the oil poses questions in the study of processed fish oils or fish products incorporated into foods. While our investigation examined both free fatty acids and freeze-dried fish powder as a health food supplement, our results cannot be simply extrapolated to all functional examples without first correcting the data for bioavailability and dose, the changed distribution of lipid classes and a change in the distribution of individual fatty acids within each class on processing.

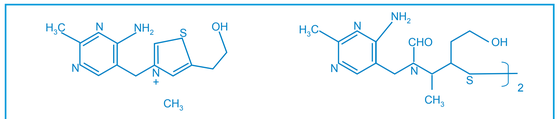

Figure 4. The active forms of Vitamin B, thiamine and thiamine disulphide. The analysis of the vitamin and provitamin can be carried out successfully by HPLC, using a ProteCol™ P C18 GP125 HPLC column, with UV detection at 210 nm or with MS detection.

Thiamine

In alternative medicine, particularly in excitable species such as the equine, thiamine (Vitamin B1) is reputed to act as an anti-anxiolytic (standing but not motion sedating) agent. As a consequence of this claim, thiamine supplementation of foods is considered by some as a functional approach to nutrition. A few attempts have been made to ascribe a mechanism of action to thiamine but these have produced equivocal results. The reputed activity of the vitamin is suggestive of central rather than peripheral activity.

Careful analysis of anecdotal evidence and quasi-clinical practices in veterinary medicine suggest that thiamine treatment is only effective when applied orally at high doses over extended periods (hypervitaminosis) or following the intravenous administration of thiamine provitamins such as thiamine disulphide (Figure 4). Urinalysis was carried out on specimens collected from horses that were calmed by either oral or intravenous treatments. Urine was either speciated by C8/SCX SPE followed by derivatisation and GC-MS analysis of the acetylated extract on a BPX5 column (Figure 5) or by analysis of the SPE fraction directly by LC-MS-MS analysis in both positive and negative ion mode in a methanol:ammonium acetate mobile phase buffered to pH 8 with ammonia. Analysis revealed that both treatments give rise to abnormally high concentrations of thiaminethiol (the monomeric form of thiamine disulphide) in the urine. The identity of the compound was confirmed by independent synthesis.

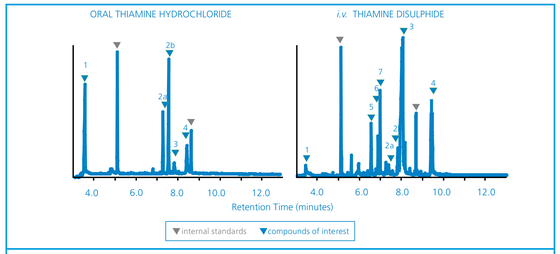

Figure 5. GC chromatograms of acetylated urine extracts from orally administered thiamine hydrochloride, and intravenously administered thiamine disulphide, analysed using a BPX5 column.

Analyses of minor components show that thiamine thiol has a propensity for further intramolecular and extramolecular reaction. The formation of the so-called diazepine artefacts was found to be a process driven by either GC injector port temperature or alternatively, as a thermodynamically preferred process during fragmentation in the mass spectrometer using LC or direct inlet. The native sulphide could be captured in negative ion LCMS under basic conditions. Discounting the diazepine compounds as physiologically available substances suggests that thiamine thiol or one of its reaction products is responsible for the anti-anxiolytic effects of thiamine.

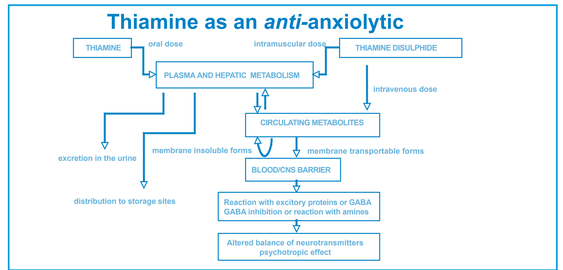

Figure 6. The actual and proposed metabolic fate of oral and intravenous doses of thiamine and thiamine disulphide.

Implications of the Study

The significant difference between thiamine cation and thiamine thiol is the likely ability of the latter to cross the blood brain barrier. Under conditions of hypervitaminosis, leading to the release of abnormally high concentrations of thiamine thiol, it is possible to propose a benzodiazepine like activity arising from the reaction of the thiol with amine neurotransmitters (e.g. dopamine) or alternatively with central receptor proteins. Using this hypothesis, the metabolic fate of hyper-dosed thiamine or thiamine disulphide is suggested in Figure 6.

Establishing claims of functional effect for thiamine fortified foods would appear to be dependant primarily on physiological concentrations of the thiamine pool rather than analysis of thiamine species in the food. Functionality may require mechanical bioassay after administration to identify efficacy in susceptible individuals.

Acknowledgements

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