REVIEW ARTICLE

Clinical Analysis, Metabolism and Bioavailability of Thiamine

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ABSTRACT
Thiamine is a component of vitamin B-complex and is used in the treatment of beriberi. The active form of the vitamin is thiamine pyrophosphate (TPP) which serves as a co-enzyme in various biochemical reactions. It is commercially available in the form of vitamin B-complex and multivitamin preparations. The clinical analysis of the vitamin and its esters is carried out by spectrophotometric, fluorimetric, high performance liquid chromatographic and flow-injection turbidimetric methods. The rates of catabolism and loss of thiamine indicate that in the absence of the vitamin, functional and clinical abnormalities occur in humans within a few weeks period. Thiamine absorption takes place in the intestine by two parallel mechanisms, i.e., saturable active transport and simple diffusion. The bioavailability of thiamine can be assessed by determining maximal thiamine concentration (C max) and its time (t max) in plasma and hemolysates, the area under concentration time curve (AUC), and thiamine excretion in 24-hr urine.

Keywords: Thiamine, stability, clinical analysis, metabolism, bioavailability.

INTRODUCTION
Thiamine (vitamin B₁) was discovered as the preventive and curative agent of the human disease, beriberi in 1926¹. The chemical name of thiamine is 3-(4’-amino-2’-methyl-pyrimidin-5’-ylmethyl)-5’-(2-hydroxyethyl)-4-methylzolium chloride and it exists in the form of hydrochloride and mononitrate salts. It contains pyrimidine and thiazole rings linked by a methylene group and the hydroxyethyl chain at position 5 of the thiazole ring, which is phosphorylated in the cell². The active form of thiamine is thiamine pyrophosphate (TPP) which serves as a co-enzyme in various metabolic reactions. Commercial multi-vitamin supplements for prevention of thiamine deficiency contain 1–5 mg/daily dose; those used for treatment of deficiency often provide 10–35 mg/daily dose³.

Physicochemical Properties and Stability
Thiamine hydrochloride is a colorless, crystalline, hygroscopic, and water-soluble vitamin. Thiamine mononitrate is less hygroscopic than the hydrochloride and is preferred for food fortification and vitamin supplements. In aqueous solution at pH 5, thiamine exhibits two absorption maxima at 235 and 267 nm and at pH 3, it has a single absorption maximum at 246 nm (ε=11,300 M⁻¹cm⁻¹). Free thiamine base is unstable and very easily oxidized. In the dry state thiamine salts are very stable even at temperatures exceeding 100 °C. Thiamine hydrochloride is acidic in aqueous solution (pH ~3.5 at 5% conc.) and below pH 5 it is stable at autoclave temperatures of 120 ºC –130 ºC, and it is not readily oxidized. At neutral pH thiamine is destroyed by oxidation especially at high temperature³. The stability of cocarboxylase (thiamine pyrophosphate chloride) in parental nutrition mixtures stored in multi-layer bags has been studied⁴. The stability of thiamine hydrochloride has been reviewed by Allwood and Kearney⁵ and Ball⁶.

Clinical Analysis
The following methods have been used for the assay of thiamine in biological samples and pharmaceutical
preparations. Thiamine is liberated from biological samples by autoclaving in 0.1 N HCl for 30 min. at about 121 °C. The free thiamine has been found to be best extracted at 108–109 °C. It can be extracted from food samples such as rice flour with 0.1 N HCl in 40% aqueous methanol for 30 min at 60 °C. To ensure complete hydrolysis of thiamine phosphates and removal of protein interference, enzyme digestion with diastase preparations containing phosphatase is used.

**Spectrometric Methods**

The UV absorption characteristics of thiamine hydrochloride may be used for its assay in pure solutions and extracted material. Thiamine absorbs at 246 nm (A 1% 1 cm = 450) in aqueous acid and this wavelength may be used for its assay purpose. However, the method is not suitable for the assay of thiamine in the presence of other UV-absorbing compounds and biological interfering material. A new graphical method based on one-dimensional wavelet transform has been proposed and tested on mixture of thiamine hydrochloride (THI) and pyridoxine hydrochloride (PYR) in the presence of strongly overlapping signals. From the UV-VIS abs. spectra a signal consisting of 1150 points corresponding to the concentration range 8-32 mg/ml for each vitamin was selected and subjected to Daubechies and biorthogonal 6.8 wavelet transforms. A zero crossing method has been applied to obtain the calibration graphs. In addition the validity of Beers law was assumed for the transformed signals. The method is suitable for analyzing the overlapping signals of compounds in mixtures without chemical pre-treatment. The simultaneous determination of thiamine (B1), pyridoxine hydrochloride (B6) and cyanocobalamin (B12) has been carried out by spectrophotometric multivariate calibration and TLC densitometry in pharmaceutical dosage forms. The vitamins were determined in their pure powder in the range 0.1-1.5 mcg (B1), 0.5-3.5 mcg (B6) and 0.1-1.5 mcg (B12). The various spectrometric methods for the assay of thiamine have been reviewed by Hashmi and Ball.

**Fluorimetric Methods**

The fluorimetric method in biological materials requires extraction of the vitamin followed by its oxidation. In the presence of potassium ferricyanide (pH ~10) it is oxidized to a highly fluorescent compound, thiochrome (excitation maximum 375 nm, emission maximum 432–435 nm). This reaction is widely used for the assay of thiamine and its phosphate esters in foods and tissue extracts. An official procedure for foods containing thiamine diphosphate requires acid digestion, enzymatic hydrolysis, purification by cation exchange open-column chromatography, conversion of thiamine to thiochrome, extraction to isobutanol and fluorescence measurement. HPLC-based assays for thiamine in foods and biological materials involve fluorescence detection. The pre-column conversion of thiamine to thiochrome gives better resolution, but may affect the working life of the column due to the use of a caustic mobile phase.

**High-Performance Liquid Chromatographic Method**

In most of the HPLC methods C18 (octadecyl silica) reverse-phase column-packaging materials have been employed. Many workers have used ion-pairing agents to interact with the positively charged nitrogen atom of thiamine for assay. A sensitive method for the post-column detection of thiamine and its phosphate esters has been reported. The procedure is based on the on-line photolysis of thiamine into photoproducts, which have a strong enhancing effect on the chemiluminescence permanganate-luminol reaction. The complete separation of the thiamines was obtained on a Reverse Phase-amine C(16) column in isocratic elution with an analysis time of less than 7 min. Under the optimum conditions analytical curves, based on standard solutions, were linear over the range 10-1000 nM for thiamine and 100-2000 nM for its mono- and di-phosphate esters. The method was successfully applied to the determination of the thiamines in pharmaceutical preparations and food supplements.

A HPLC method has been developed for the simultaneous determination of thiamine and its
phosphate esters (cocarboxylase) in whole blood to determine the thiamine status in normal and pathophysiological conditions. The detection limits are between 0.067-0.09 pg/l (0.160-1.822 f mol/l)\textsuperscript{15}. Pongothai et al. have developed a simple, precise reverse-phase HPLC method and validated it for the determination of vitamin B\textsubscript{1}, B\textsubscript{6} and B\textsubscript{12} in multivitamin capsules. The RSD of the method was in the range of 0.7-1.7%. The mean recoveries were found to be in the range of 99.8-100.2\%\textsuperscript{16}. The simultaneous determination of thiamine hydrochloride along with riboflavin, niacin amide, pyridoxine, cyanocobalamin and folic acid in multi-vitamin tablets has been reported\textsuperscript{17,18}.

**Flow Injection Turbidimetric Method**

A simple flow injection system is proposed for the assay of thiamine. It is based on the precipitation reaction of thiamine with silicotungstic acid in acidic medium to form thiamine silicotungstate that is measured at 420 nm. The calibration graph is linear in the thiamine concentration range of $5.0 \times 10^{-5}$ to $3.0 \times 10^{-4}$ M with a detection limit of $1.0 \times 10^{-5}$ M. The RSD for 10 successive measurements of $1.0 \times 10^{-4}$ M and $2.5 \times 10^{-4}$ M thiamine were less than 1%. The method can be applied to the assay of thiamine in pharmaceutical formulations and biological fluids\textsuperscript{19}.

**Metabolism**

The rates of catabolism and loss of thiamine in the body indicate that if the dietary intake of the vitamin falls to near zero, the functional and clinical abnormalities become apparent in humans within a few weeks and it is more rapid than that of most of the other micronutrients. It has been reported that about half of the total amount of thiamine in the body is present in muscle. The total amount of thiamine in a well nourished adult human has been found to be about 30 mg, of which about four-fifths occurs as the enzyme co-factor, thiamine diphosphate.

The diphosphate moiety of adenosine triphosphate (ATP) is required for the synthesis of thiamine diphosphate from free thiamine by the action of thiamine pyrophosphokinase. The metabolism of thiamine phosphate occurs either by dephosphorylation to form thiamine monophosphate and is catalyzed by thiamine pyrophosphatase. Further phosphorylation may occur to give thiamine triphosphate. This reaction is catalyzed by thiamine diphosphate-ATP phosphoryltransferase\textsuperscript{20}. Free thiamine can be converted, to a limited extend, to thiamine monophosphate by alkaline phosphates in the presence of phosphate donors\textsuperscript{21}.

Thiamine phosphate esters present in food are transformed to free thiamine by digestive enzymes (phosphatases) before the vitamin is absorbed. The excess vitamin is metabolized or degraded by the gut flora of the large bowel\textsuperscript{2}.

**Bioavailability**

The free thiamine and monophosphate are absorbed by the intestinal mucosa. The transportation of thiamine is more efficient than its monophosphate\textsuperscript{21}. In the human and animal species thiamine absorption in the intestine takes place by two parallel mechanisms\textsuperscript{2}.

1. Saturated active transport which occurs at low luminal thiamine concentrations (<1.25 μM), and
2. Simple diffusion at higher luminal thiamine concentrations.

In a study of human intestinal biopsies\textsuperscript{22}, the low concentration saturable pathway has been found to exhibit Michaelis-Menten-type kinetics, with an apparent $k_m$ of 4.4 μM for thiamine. The active transport process is competitively inhibited by all three types of thiamine analogues, namely, oxythiamine, pyrithiamine and amprolium\textsuperscript{2}.

In a multiple change-over study the bioequivalence of three thiamine preparations, used therapeutically as neurotropic agents for the treatment of polyneuropathies, was tested in seven volunteers. After ingestion of a single dose of either 100 mg benfonatiamin (S-benzoylthiamine-o-monophosphate), fursultiamin (thiamintetrahydrofururyldisulfide) or
thiaminedisulfide, thiamine blood levels were analyzed for a 10-hours period. Thiamine was measured by HPLC after percolum derivatization to thiochrome. The maximal thiamine concentration (C_max) and its time (t_max) in plasma and hemolysate, the area under concentration time curve (AUC), and thiamine excretion in 24-hours urine were assessed as criteria of bioavailability. All biokinetic data demonstrated a significantly improved thiamine bioavailability from benfotiamin compared with the other preparations.

The pharmacokinetics of two therapeutically used thiamine preparations was assessed in a comparative study with 20 end-stage renal disease (ESRD) patients. After a single oral dose of either 100 mg benfotamin (S-benzoyltiamine-o-monophosphate, BTMP) or 100 mg thiamine mononitrate (TN), blood levels of thiamine phosphate esters were analyzed by HPLC after precolumn derivatization to thiochrome phosphate esters for a 24-hour period. The pharmacokinetics parameters AUC 0-24 hr, C_max and t_max of the benfotamin group in whole blood and plasma exceeded than those significantly those in the TN group. In addition, a high transfer rate to thiamine diphosphate (TDP) was observed in the patients after ingestion of BTMP. The TDP concentration in the whole blood increased by 2.6 and 1.4 times from baseline levels to C_max in the BTMP and TN groups, respectively.

DISCUSSION

The present review provides information on certain aspects of thiamine (vitamin B_1), including the physicochemical properties, stability, clinical assay methods, metabolic processes and bioavailability from commercial vitamin B-complex preparations. The UV absorption and fluorescence emission characteristics of thiamine may directly be utilized for assay purpose or for detection in HPLC assays. The current approach in the clinical assay of thiamine is by HPLC using fluorescence detection based on pre-column oxidation of thiamine to thiochrome and measurement of its fluorescence. In biological systems thiamine exists in the form of its pyrophosphate (TPP) ester which serves as a coenzyme in several metabolic reactions. The bioavailability of thiamine in pharmaceutical preparations can be evaluated by the determination of C_max, t_max, AUC and thiamine excretion data in 24 hours. These data could provide information on the bioequivalence of thiamine in different pharmaceutical preparations.

REFERENCES

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