Physiological Functions, Role in Metabolism and Clinical Analysis of Riboflavin

Tania Mirza, Sadia Hafeez Kazi, and Iqbal Ahmad

ABSTRACT

Riboflavin (Vitamin B2) is the precursor in the biosynthesis of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The metabolic functions of riboflavin are carried out by these coenzymes, which participate in numerous vital oxidation-reduction processes. Flavoproteins catalyze dehydrogenations, hydroxylation, oxidative decarboxylations, dioxygenation and reductions of oxygen to hydrogen peroxide reactions. The powerful anti-oxidant activity of the vitamin is derived from its role as a precursor to FMN and FAD. A major protective role against lipid peroxides is provided by the glutathione redox cycle. Glutathione peroxidase breaks down reactive lipid peroxides. The enzyme requires \( \gamma \)-Glutamyl-L-cysteine-glycine (GSH) that is generated from its oxidizing form glutathione disulfide (GSSG) by the FAD containing enzyme glutathione reductase. The administration of riboflavin may be helpful in certain inborn errors of metabolism. The riboflavin deficiency may have a beneficial role in malaria. The clinical analysis of riboflavin is largely carried out by high-performance liquid chromatographic methods (HPLC)

Keywords: Riboflavin, deficiency, clinical Analysis, physiological functions, metabolism.

Introduction

The earliest scientific studies on the prevention of a deficiency state and other factors by riboflavin were made by McCollum and Kennedy in 1916. In later studies a heat stable fraction was isolated from different sources including milk, egg and liver. The fraction contained a yellow green fluorescent growth factor. This on purification was named riboflavin by Emmett and Luros in 1920. And is now called vitamin B2. Warburg and Christian in 1932 described the physiological role of the yellow growth factor called as “old yellow enzyme”. The enzyme was composed of an apoenzyme and a yellow cofactor as yellow coenzyme prosthetic group. The coenzyme contained as isoalloxazine ring4 and a phosphate containing side chain5. The first coenzyme formed from riboflavin is riboflavin-5'-phosphate, also called flavin mononucleotide (FMN)6 and the second coenzyme formed is flavin adenine dinucleotide (FAD)7. The role of riboflavin in health8, the regulation of metabolism9, the inborn errors of riboflavin metabolism10, nutrition11 and specific functions12 have been reviewed in detail.

Metabolism

FMN and FAD function as coenzymes for enzymes involved in a wide variety of reactions in intermediary metabolism. There are also other forms of FAD that are covalently linked from the 8-x position of the isoalloxazine portion of the flavin via N(1) or N(3) of histidy1 or the S of cysteinyl residues within specific enzymes that have a number of significant roles in metabolism13. The mammalian enzymes including sarcosine dehydrogenase, Succin dehydrogenase, monoamine oxidase and L-gulonolactone oxidase are covalently bound of flavins12. Microsomal NADPH-cytochrome P450 reductase is the first mammalian enzyme that has been found to contain both FMN and FAD as coenzyme in equimolar ratios. In addition nitric oxide synthase and methionine synthase are known to contain FMN and FAD as coenzymes14,15. The important role of riboflavin in fat metabolism has shown that in certain inborn errors of metabolism there may be a need of therapeutic administration of riboflavin. In the deficiency of acyl-CoA dehydrogenase, infants with recurrent hypoglycemia, lipid storage myopathy and
increased urinary excretion of organic acids, riboflavin supplementation has lead to rapid clinical improvement. The genetic disorder referred to as riboflavin-responsive, multiple acylcoenzyme A dehydrogenase deficiency results from lipid storage myopathy and decreased B-oxidation. An uncoupling protein (UCP3) is increased in this disorder and may be involved in the associated metabolic abnormalities. At present, Type B lactic acidosis occurs in association with HIV treatment and also responds to riboflavin. Flavoproteins catalyze dehydrogenation reactions as well as hydroxylation, oxidative decahydroxylations, dioxygenations and reductions of oxygen to hydrogen peroxide. This indicates that many different kinds of oxidative and reductive reactions are catalyzed by flavoproteins. Flavin coenzymes are involved in the metabolism of folic acid, pyridoxine, vitamin K, niacin and vitamin D.

Physiological functions
The major function or riboflavin is in the form of a precursor of the flavin coenzymes, FMN and FAD and also the covalently bond flavins. These coenzymes catalyze numerous oxidation-reduction reactions. Other functions of riboflavin include drug, steroid and lipid metabolism. The redox functions of flavin coenzymes involve both 1-electron transfer and 2-electron transfer from a substrate to the flavin coenzymes.

Antioxidant activity
Riboflavin exhibits powerful antioxidant activity that is derived from the role of the vitamin as a precursor to FMN and FAD. Glutathione redox cycle provides a major protective role against lipid peroxides. This reaction is carried out in the presence of y-Glutamyl-L-cysteine-glysine (GSH) which is generated from its oxidized form, GSSG, by the FAD-containing enzyme glutathione reductase. In this case riboflavin nutrition could be critical in the regulation of the rate of inactivation of lipid peroxides. Riboflavin deficiency has been associated with increase hepatic liver peroxidation and riboflavin supplementation controls this process.

Riboflavin homocysteine
An important role of riboflavin in its involvement in its regulation of homocysteine metabolism. It takes part in the pathogenesis of vascular diseases. Methylcobalamin is also a coenzyme in this enzymatic reaction. Vitamin B is involved in the inactivation of homocysteine as a coenzyme for two digestive enzymes cystathionine B-synthase and cystathioninase. The efficient utilization of dietary folic acid requires adequate amount of riboflavin in nutrition. It has been reported that the state of riboflavin nutrition controls homocysteine metabolism in patients who are homozygous individuals. The patient with this genotype would respond more rapidly to riboflavin supplementation than those individuals without the genetic variation.

Riboflavin and malaria
The normal riboflavin nutritional status has been associated with high levels of parasitemia in human infants suffering from malaria. Iron and vitamin supplementation has been found of result in increased malaria parasitemia. Thus riboflavin deficiency may be protective against malaria in humans. The mechanism by which riboflavin deficiency inhibits malaria parasitemia is not known. There may be effects on the redox status of erythrocytes that are considered as an important determinant of the growth of malarial parasites. These results suggest that parasites may have a higher requirement for riboflavin than the host erythrocyte.

Photosensitization
The photosensitizing property of riboflavin may have some potential risks. Phototherapy in vitro degrades DNA and increases lipid peroxidation, which may have implications for carcinogenesis, mutagenesis and other disorders. When rat erythrocytes are irradiated in the presence of FMN, an increase potassium loss occurs. In the topical administration on riboflavin
tryptophan adduct accelerate the photoaddition of melanin. Since vinca alkaloids undergo photosensitization by riboflavin, the results of efficacy of testing of cytotoxic drugs may not be reliable.  

Clinical analysis
High Performance Liquid Charomatography (HPLC) has been widely used for the clinical analysis of riboflavin, FMN and FAD in biological materials. In the HPLC assay of riboflavin in urine the limit of its fluorimetric detection is mcg/L.  Riboflavin, FMN and FAD in plasma and ruine have been assayed by fluorimetric detection. The determination of riboflavin, thiamine and pyridoxine in whole blood and serum has been carried out by HPLC using ultraviolet and fluorescent detection. Riboflavin and flavor coenzymes (FMN and FAD) have been determined in plasma by fluorescence detection. The limits of detection for riboflavin are 3 nmol/liter and for the flavo coenzymes, 9 nmol/L. The determination of riboflavin and riboflavin cofactors in plasma by HPLC has also been reported.  

A reversed-phase ion-pair HPLC method has been developed in validated for the routine analysis of riboflavin, thiamine mononitrate and pyridoxine hydrochloride in multi-vitamin with mineral tablets using a Hypersil C18 column and ultraviolet detection at 280 nm. The method was linear in the range of 2.5 to 90 mcg/mL. The simultaneous determination of riboflavin along with other B vitamins in multi-vitamin tablets by paired-iron reverse-phase HPLC has been reported. A rapid determination of riboflavin in plasma by HPLC method has been performed. The method has been validated for linearity, limit of quantification, accuracy, precision and interference. It is accurate and correlates well (R2= 0.993) to expected concentration of spiked pooled plasma samples. The reference interval established by non-parametric analysis is 6.7-50.1 nmol/L. The detection was carried out fluorometrically at 520 nm. Ion pair reversed-phase HPLC has also been applied to the simultaneous determination of vitamin B1, B2, B6, B12 and C, and nicotinamide and folic acid in vitamin preparations. An early application of high-speed liquid chromatography to the analysis of riboflavin in multivitamin preparations has been reported. The vitamin is detected at 254 nm with a reproducibility of 27.23 to 103.45% of the labeled amount of riboflavin. HPLC methods have been used for the analysis of riboflavin and metabolites using fluorescence detection.

Discussion
The review throws light on the physiological functions, role in metabolism and clinical analysis of riboflavin. Riboflavin plays its biological role in the form of the coenzymes, (FMN) and (FAD). These coenzymes are involved in a wide variety of reactions taken place in intermediary metabolism. The planner isaloalzazine ring system is the basic structure of riboflavin, FMN and FAD. Riboflavin performs several physiological functions among which the major function of the vitamin is to serve as a precursor of the flavin coenzymes, FMN and FAD and covalently bound flavins. These coenzymes catalyze numerous oxidation-reduction reactions in the physiological systems. Since FAD is part of the respiratory chain, riboflavin has a central role in energy production. Some other important functions of riboflavin include drug and steroid metabolism, in conjunction with cytochrome P450 enzymes, and lipid metabolism. The redox functions of flavin coenzymes are based on one-electron and two-electron transfers from the substrate to the flavin coenzymes. As a precursor to FMN and FAD, riboflavin exhibits a powerful antioxidant activity. Riboflavin deficiency is reported to be associated with compromised oxidant defense and the supplementation of riboflavin and its active analogues improves oxidant status. Riboflavin deficiency is associated with malaria and certain inborn metabolic error. HPLC is the most widely used technique for the clinical analysis of riboflavin alone and in the presence of its coenzymes, other vitamins and metabolites.
REFERENCES


05. Theorell, H. Reinderstellung (Kristallisation)des gelbenAtmungsfermentes und die reversible Spaltung desselben. Biochem., Z. 1934; 272: 155-156

06. Theorell, H. Die freie Eiweissknmponente des gelben Ferments und ihrer Kupplung mit Lacto-flavinphosphorsaure. Biochem., 1937; 290:293-308


40. Ahmad, I. and Usmanghani, K. Analysis of medicinal compounds and plant drugs. Research Institute of Indusyunic Medicine, Karachi. 2003; p. 60.