Nitrogen-stimulated Orotic Acid Synthesis and Nucleotide Imbalance

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Abstract

Orotic acid, first discovered in ruminant milk, is an intermediate in the pyrimidine biosynthesis pathway of animal cells. Its synthesis is initiated by the formation of carbamoyl phosphate (CP) in the cytoplasm, with ammonia derived from glutamine. Ureotelic species also form CP in the first step of urea synthesis in liver mitochondria. For that, ammonia is derived from tissue fluid. When there is insufficient capacity for detoxifying the load of ammonia presented for urea synthesis, CP leaves the mitochondria and enters the pyrimidine pathway, where orotic acid biosynthesis is stimulated, orotic acid excretion in urine then increases. Orotic acid synthesis is abnormally high with hereditary deficiencies of urea-cycle enzymes or uridine monophosphate synthase. It is also elevated by ammonia intoxication and during feeding of diets high in protein, high in lysine with respect to arginine, or deficient in arginine, ornithine, and citrulline. Rats fed 1% orotic acid or diets deficient in urate cycle amino acids develop fatty livers, which has not been demonstrated in other species. Humans consuming 6 g of orotic acid daily have not shown adverse effects. Rats fed 1% orotic acid or arginine-deficient diets also showed more and larger foci positive for γ-glutamyl transpeptidase and more liver tumors after administration of carcinogens and partial hepatectomy. Orotic acid feeding was also associated with the tendency for development of larger mammary tumors induced by chemical carcinogens in rats and with development of urinary bladder calculi containing high concentrations of orotic acid in mice. Conditions that raise tissue orotic acid change purine-pyrimidine ratios. It is unknown whether tissue orotic acid concentrations of orotic acid in mice. Conditions that raise tissue orotic acid concentrations of orotic acid may be associated with cancer by intrarectal administration of ammonium acetate.

Introduction

Orotic acid, an intermediate in pyrimidine biosynthesis, which is its only known biological source (Fig. 1), was discovered in bovine milk by Biscaro and Belloni in 1905 (1). Pyrimidine biosynthesis starts with the formation of CP, which is also an intermediate in the initial steps of urea biosynthesis. The formation of CP, which is essential for pyrimidine synthesis, occurs in the cytosol of all tissues in a reaction catalyzed by carbamoyl phosphate synthetase II, whereas CP destined for urea synthesis is formed in the liver mitochondria of ureotelic species in a reaction catalyzed by carbamoyl phosphate synthetase I. Tissue fluid is the source of ammonia for CP synthesis in mitochondria and glutamine is the source in cytosol. The committed step in the synthesis of pyrimidines is the formation of carbamoyl aspartate, catalyzed by aspartate carbamoylase. This is followed by cyclization to form dihydroorotate and dehydrogenation to yield orotate. Orotate, a precursor of nucleic acid pyrimidines, reacts with 5-phosphoribosyl pyrophosphate to form orotidine-5′-phosphate, which then undergoes decarboxylation to yield UMP, the direct precursor of all other pyrimidine nucleotides. All enzymes of this pathway are cytoplasmic except for dihydroorotic acid dehydrogenase, which is bound to the inner mitochondrial membrane. The cytoplasmic enzymes reside in two separate multifunctional complexes. One contains carbamoyl phosphate synthetase II, aspartate transcarbamylase, and dihydroorotase, whereas the other includes orotate phosphoribosyltransferase and orotidine-5′-phosphate decarboxylase (2, 3). A deficiency of the latter two enzyme activities results in accumulation of orotate and a profound rise in its excretion in the urine, a condition known as hereditary orotic aciduria (4). This bifunctional protein complex with its two enzyme activities is also referred to as UMP synthase.

A severe deficiency of UMP synthase elevates urinary orotic acid excretion in humans to 1500 mg/day, compared with the usual 2.5 mg/day. Megaloblastic anemia and growth retardation are accompanying conditions (4). Robinson et al. (5) discovered an analogous condition in Holstein cows, which show a partial deficiency of UMP synthase in their RBC. This hereditary condition exhibits an autosomal recessive mode of inheritance, with heterozygotes showing high concentrations of orotate in their milk and urine and no other known detrimental consequences. The homozygous recessive genotypes die in utero near the 40th day after conception (6).

Ammonia-induced Orotic Aciduria

Kesner (7) first reported that injections of ammonium salts raised urinary orotic acid excretion by rats. Ureotelic species also show elevated orotic acid excretion with ammonia intoxication from hereditary urea cycle deficiency (8); injections of toxic doses of urease (9) or amino acids (10); diets lacking the three urea cycle amino acids ornithine, citrulline, and arginine (11); or diets containing excess lysine, which is an antagonist of arginine (12). Elevation of orotic acid excretion and fatty liver caused by excess lysine can be overcome by feeding adenine or allopurinol (13). Recent studies also show that there are 2–3-fold elevations in urinary orotate in concert with high intakes of dietary protein in otherwise complete diets consumed by other species (14, 15). Increasing the load of tissue ammonia for detoxification by the urea cycle increases the formation of CP by liver mitochondria of ureotelic species. When CP synthesis exceeds the capacity for its use in subsequent steps of urea synthesis, CP escapes into the cytosol and the synthesis of orotate is increased in the cytosolic pyrimidine pathway (14, 16). Because it is water soluble and readily excreted by the kidney, levels of orotate rise rapidly in the urine as its synthesis increases. Its level of excretion has been suggested as an indicator of ammonia intoxication and thereby an indirect measure of the balance between tissue ammonia concentrations and the capacity for urea synthesis. The quantities of orotate excreted are not sufficient to play a significant role as a form of excreted nitrogen (17).

Effects of Orotic Acid Consumption

Milk of ruminants contains higher concentrations of orotic acid than does milk from other species, where it normally ranges from 0 to 5 µg/ml and approximates the concentrations in other tissues and fluids. Orotic acid in milk of normal cows is elevated

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3 The abbreviation used is: CP, carbamoyl phosphate.
The pathway of pyrimidine nucleotide biosynthesis is shown in Fig. 1. Orotic acid intake is generally considered in early lactation and then stabilizes around 80 μg/ml, or about 0.1–0.2% of the nonfat total solids. Bovine milk and other dairy products are the only human foods known to contain substantial quantities. In 1955 Standerfer and Handler (19) showed that feeding orotic acid caused fatty livers. This response, which appears specific for rats, has served as a stimulus for research on the biological effects of orotic acid, because of the importance of cow milk in the diet of infants and other humans. On the basis of present evidence, the risks to humans from usual levels of orotate consumption appear to be minimal, and the mechanism underlying the development of fatty livers in rats is unknown. Fallon et al. (20) found that i.v. injection of orotic acid into humans caused prompt urinary excretion of orotate and urate, and they suggested that the two metabolites compete for renal reabsorption. Orally administered orotic acid did not increase excretion of either compound. Kelley et al. (21) showed increased urinary uric acid after orotic acid ingestion by humans, but they did not determine orotic acid excretion. They found that orotic acid taken orally decreased serum uric acid in their patients whether serum uric acid was normal or elevated. They also found that 6 g of orotic acid taken daily for 4–7 days led to modest decreases in plasma lipids and cholesterol.

Robinson and Dombrowski (22) studied the pharmacology of orotic acid in 29 healthy adult volunteers who had consumed 0–6 g at weekly intervals for 4 consecutive weeks. After ingestion of 6 g, their excretion of orotate and urate increased significantly, but creatinine and urea excretion remained unaffected. Excretion of orotate was proportional to the dose and, was generally complete within 8 hours, and the excretion pattern for urate was similar. Mean excretion of orotate was 10.5% of the dose, varied from 1% to 26%, and was unrelated to gender. As reported by Kelley et al. (21), blood cholesterol decreased after orotic acid ingestion. Orotate excretion after the ingestion of placebos averaged 2.52 ± 0.11 mg/day, which is between the values of 1.5 and 2.8 mg/day reported by Tax et al. (23) and Visek et al. (24), respectively. As reported by others, excretion was similar whether expressed as mg/24 h or mg/g creatinine. Durschlag and Robinson (25) found that rats excreted an average of 7.6% of an ingested tracer dose of [14C]orotate, and mice 27.4%, in 24 h.

Triglycerides are the form of fat deposited in the liver of rats fed 0.5% or more orotic acid in their diet. This has been attributed to decreased secretion of lipoproteins, with accumulation of nascent lipoproteins in the Golgi apparatus and an accompanying depression in blood lipids. Paired-feeding studies by Durschlag and Robinson (25) showed that the depression was related to orotate intake and not to other dietary conditions. Adding uracil plus cytosine, guanine, hypoxanthine, or xanthine for 7 days did not prevent the condition (26). Valli et al. (27) fed diets with 1% orotic acid to mice, chicks, and monkeys, and Bloomfield et al. (28) fed 2% orotic acid to chicks for 4 weeks. Neither group of investigators reported accumulation of hepatic fat in their experimental animals. Durschlag and Robinson (25) examined the consequences of feeding 1% orotic acid, for up to 30 days, in a basal diet common to mice, guinea pigs, hamsters, pigs, and rats, under conditions of paired feeding. Only pigs and rats showed increased liver weight and only rats showed hepatic steatosis. None of the other species fed the same basal diet, nor monkeys fed a different basal diet containing orotic acid, showed changes in liver fat. Rats and mice showed a depression in adenosine-uridine nucleotide ratios, presumably because of competition for 5-phosphoribosyl-pyrophosphate, an intermediate required for orotate metabolism and purine nucleotide synthesis. The depression was greater in rats than in mice. Kelley et al. (21) reported similar results in human subjects, with depletion of 5-phosphoribosyl-pyrophosphate concentrations in the RBCs of the subjects after they had consumed 6 g of orotic acid.

Orotic Acid in Tumor Promotion

Original observations of the promotion of chemically induced liver cancers by orotic acid feeding were described by Sarma and associates (29, 30). Male Fischer 344 and Sprague Dawley rats have shown more and larger foci positive for γ-glutamyl transpeptidase, which ultimately develop into hepatic nodules and hepatocellular carcinoma. These have occurred after administration of 1,2-dimethylhydrazine or benzo(a)pyrene and partial hepatectomy (31). In studies at another laboratory, rats fed 1% orotic acid also tended to show larger chemically induced mammary tumors (32). The precancerous liver lesions advance to histologically defined carcinomas and appear not to be strain or carcinogen specific. Possible mechanisms underlying the cancer-promoting actions of orotic acid are discussed in the contribution by Manjeshwar and associates in this symposium (33).

The considerable variation in the rate of excretion of ingested orotate between species may be a determinant of the kinds of pathological changes that ensue. For example, the relatively low excretion rate, which is <8% in 24 h for rats, may enhance tumor promotion in the liver, which is associated with greater depression in purine-pyrimidine ratios in rats than in mice. Mice excreted 27% of their labeled orotic acid in the urine after p.o. administration (25). Albino mice developed a high incidence of urinary bladder stones of high orotate content after consuming a semipurified diet containing 1% orotic acid (34).

Orotic acid synthesis is enhanced by increasing protein intake, and high protein intake is correlated with enhanced cancer incidence. All amino acids are potential sources of ammonia, and more ammonia is released by metabolic processes when protein intakes rise. Ammonia shortens cell life span and increases the protein and RNA content of the intestinal mucosa (17). High-protein, high-fat diets increase ammonia concentrations in the lumen and cell proliferation in the mucosa of the

\[ \text{T. S. Elliott et al., unpublished observations.} \]

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colon of rats (35). It was also shown that ammonia causes damage of the colonic mucosa (36) and that intrarectal infusions of ammonium acetate increase the incidence and dysplasia of chemically induced carcinoma in rats (37). Whether ammonia causes these changes by direct toxic action on cells or whether the changes are secondary to changes in nucleotide synthesis or other metabolic processes caused by ammonia remains to be determined.

References

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