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Rescue from neonatal death in the murine model of hereditary tyrosinemia by glutathione monoethylester and vitamin C treatment

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Abstract

Hereditary tyrosinemia type 1 (HT1) is a recessive disease caused by a deficiency of the enzyme fumarylacetoacetate hydrolase (FAH) that catalyzes the conversion of fumarylacetoacetate (FAA) into fumarate and acetoacetate. In mice models of HT1, FAH deficiency causes death within the first 24 h after birth. Administration of 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3 cyclohexanedione (NTBC) prevents neonatal death in HT1 mice, ameliorates the HT1 phenotype but does not prevent development of hepatocellular carcinoma later on. FAA has been shown to deplete cells of glutathione by forming adducts. We tested whether a combination of a cell membrane permeable derivative of glutathione, glutathione monoethylester (GSH-MEE) and vitamin C could provide an alternative effective treatment for HT1. GSH-MEE (10 mmol/kg/j)/vitamin C (0.5 mmol/kg/j) treatment was given orally to pregnant/nursing female mice. While $FAH^{-/-}$ pups died in absence of treatment, all $FAH^{-/-}$ pups survived the critical first 24 h of life when the mothers were on the GSH-MEE/vitamin C treatment and showed normal growth until postnatal day 10 (P10). However, after P10, pups showed failure to thrive, lethargy and died around P17. Thus, GSH-MEE/vitamin C supplementation could rescue the mice model of HT1 from neonatal death but it did not prevent the appearance of a HT1 phenotype in the second week after birth.

Keywords: Hereditary tyrosinemia type 1; Fumarylacetoacetate hydrolase; Glutathione; NTBC

Hereditary tyrosinemia type 1 (HT1; McKusick 27670) is a severe childhood disease caused by a deficiency of the last enzyme of the tyrosine catabolic pathway, fumarylacetoacetate hydrolase (FAH), Fig. 1 [1]. HT1 is an autosomal recessive trait particularly frequent in Eastern Quebec, which has the highest worldwide incidence (1:1850 births) and carrier rate (1:22) due to a founder effect [2,3]. HT1 presents two main clinical forms, acute and chronic. The acute form is characterized by hepatic dysfunction associated with cirrhosis, hepato- and splenomegaly leading to death in the first months of life. In the chronic form, patients show renal dysfunction, vitamin D-resistant rickets, growth retardation, gradual liver alterations, cirrhosis and liver cancer. Hepatocellular carcinoma (HCC) has been reported in 37% of HT1 patients over two years prior to NTBC therapy [4]. Dietary restriction, while beneficial at the beginning, does not fully prevent progressive liver damage and renal dysfunction. Orthotopic liver transplantation allows restoration of liver functions, although renal abnormalities can sometimes persist due to remaining FAH deficiency in kidneys. NTBC (2-(2-nitro-4-trifluoro-

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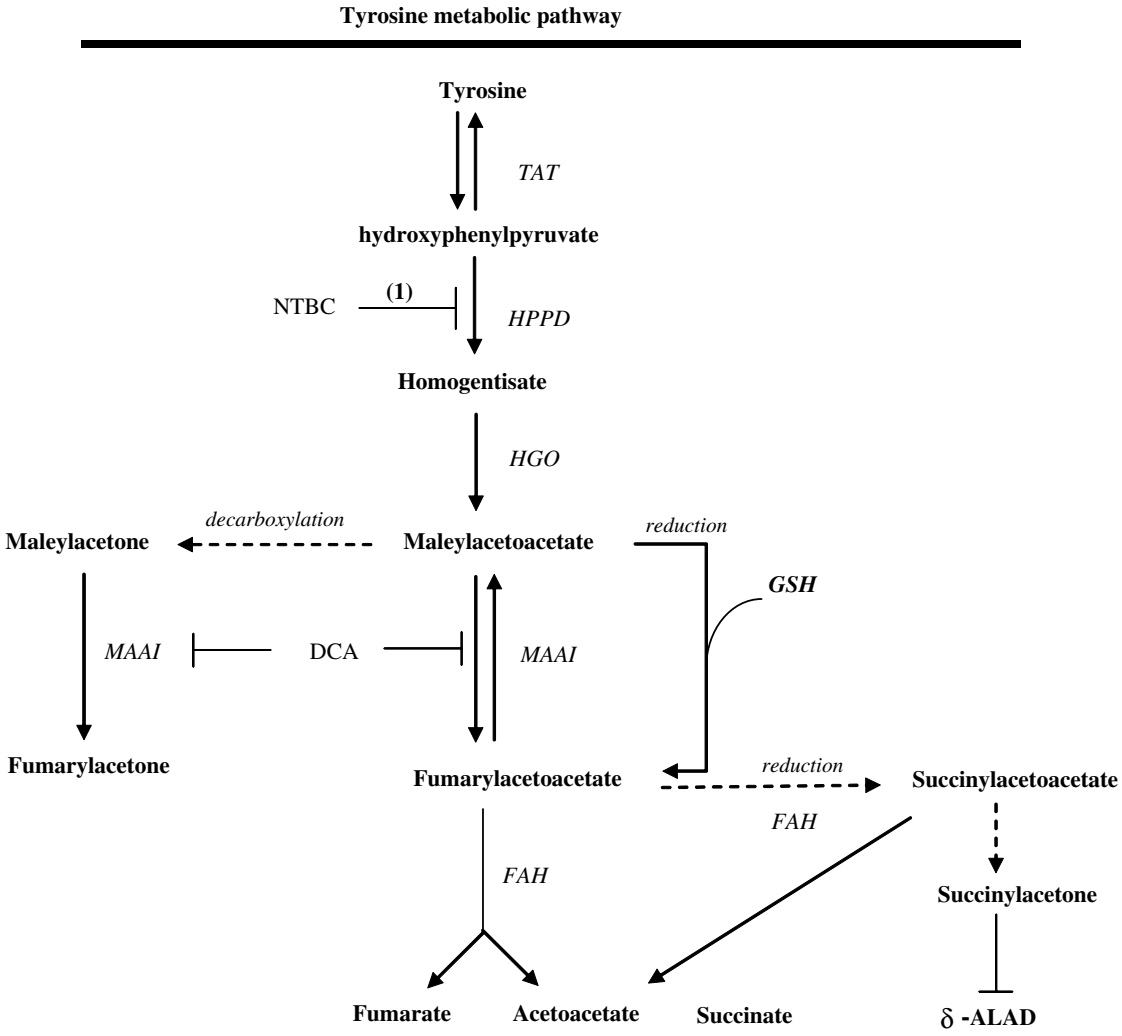

Fig. 1. The five enzymatic reactions of the tyrosine catabolism. Inhibition step of NTBC (1) and the GSH-dependent non-enzymatic bypass for MAA transformation to FAA (2) are indicated. Dotted lines represent putative pathways with unknown enzymes. TAT, tyrosine aminotransferase; HPPD, 4-hydroxyphenylpyruvate dioxygenase; HGO, homogentisate dioxygenase; MAAI, maleylacetoacetate isomerase; FAH, fumarylacetoacetate hydrolase; NTBC, 2-(2-nitro-4-trifluoromethylbenzoyl) 1,3-cyclohexanedione; δ-ALAD, δ-aminolevulinic acid dehydratase.

methylbenzoyl)-1,3 cyclohexanedione), an inhibitor of the upstream enzyme 4-hydroxy phenylpyruvate dioxygenase (HPPD, Fig. 1), rapidly restores normal hepatic functions and has been successfully used in HT1 patients since 1992 [5–7]. However, the efficiency of NTBC over time as a HCC preventing agent is uncertain since NTBC-treated $FAH^{-/-}$ mice develop dysplasia after 7 months and 13–17% develop HCC after 18–24 months, even when using a most stringent therapy, e.g., a higher NTBC dose (4 mg/kg/day) plus dietary tyrosine restriction [8,9]. In human, some patients do not respond to NTBC and there have been several reports of HCC in HT1 patients on NTBC treatment [7,10–13]. Thus, search for alternative and/or complementary HT1 treatments in counteracting the effects of toxic accumulated metabolites remains a highly relevant challenge.

HT1 patients accumulate the toxic metabolites, fumarylacetoacetate (FAA), maleylacetoacetate (MAA), and succinylacetone (SA). The therapeutic routes applied to HT1 are essentially based on the inhibition of the tyrosine catabolic pathway upstream of the enzymatic defect. Unlike NTBC, which inhibits HPPD, blocking the proximal upstream enzyme maleylacetoacetate isomerase (MAAI) with the inhibitor dichloroacetate (DCA) (Fig. 1) did not prevent the HT1-associated damage in a mouse model of the disease [14]. In cultured cells, FAA shows mutagenicity and induces apoptosis, both of which are potentiated by GSH depletion [15–17]. FAA by itself decreases intracellular GSH contents [15]. Both FAA and its derivative SA rapidly react with GSH to form stable adducts [18,19]. Decreased GSH content has been found in the liver and blood of some HT1 patients and is associated with poor prognosis [20,21]. Thus, tissue damage caused by FAA toxicity in HT1 may be potentiated by GSH depletion.

Since one of the effects of the toxic compound FAA is to decrease GSH, a major cellular antioxidant, we reasoned that increasing the pool of GSH by supplementation might prevent some of the harmful manifestations of the HT1 phenotype. Various reports [15–17,19] have suggested that