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The effects of partial thiamin deficiency and oxidative stress (i.e., glyoxal and methylglyoxal) on the levels of α-oxoaldehyde plasma protein adducts in Fischer 344 rats

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Abstract We hypothesized that in marginal thiamin deficiency intracellular α-oxoaldehydes form macromolecular adducts that could possibly be genotoxic in colon cells; and that in the presence of oxidative stress these effects are augmented because of decreased detoxification of these aldehydes. We have demonstrated that reduced dietary thiamin in F344 rats decreased transketolase activity and increased α-oxoaldehyde adduct levels. The methylglyoxal protein adduct level was not affected by oral glyoxal or methylglyoxal in the animals receiving thiamin at the control levels but was markedly increased in the animals on a thiamin-reduced diet. These observations are consistent with our suggestion that the induction of aberrant crypt foci with marginally thiamin-deficient diets may be a consequence of the formation of methylglyoxal adducts.

Keywords: α-Oxoaldehydes; Methylglyoxal; Glyoxal; Oxidative stress; Thiamin; Protein adducts

1. Introduction

Thiamin (vitamin B_1) in its diphosphate form (TDP) is an important coenzyme for transketolase (TK), pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and the branched-chain α-ketoglutarate dehydrogenase complex. These enzymes are involved in the maintenance of NADPH levels and carbohydrate metabolism in the cell (Scheme 1) [1,2]. Thornalley et al. [3] have shown that accumulation of methylglyoxal (MG, an α-oxoaldehyde) and triose phosphates in human red blood cells incubated under hyperglycemic conditions can be prevented by thiamin. Moreover, Thornalley's group has recently shown that high dose thiamin and benfotiamine (lipophilic derivative of thiamin) therapy in diets administered to streptozotocin induced diabetic rats prevented incipient diabetic nephropathy induced by reactive α-oxoaldehyde [4].

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Abbreviations: MG, methylglyoxal; TK, transketolase; TPP, thiamin pyrophosphate; RBC, red blood cells; ACF, aberrant crypt foci; AGEs, advanced glycation end-products

MG is formed by the non-ezymatic elimination of phosphate from the triosephosphates glyceraldehyde-3-phosphate and glycerone phosphate, as well as enzymatically from dihydroxyacetone phosphate catalyzed by the allosterically regulated MG synthase [5,6]. It is also formed via ketone body metabolism from acetone, and by the metabolism of threonine [7]. Glyoxal (MG derivative), however, is a product of lipid peroxidation, ascorbate autoxidation, oxidative degradation of glucose and glycated proteins [8]. MG and glyoxal are bifunctional alkylating agents that react with free amino and thiol groups of biomolecules, resulting in the formation of advanced glycation end-products (AGEs). Glyoxal and MG react non-enzymatically and reversibly with lysine, arginine and cysteine residues of proteins [9,8]. Irreversible reactions with lysine and arginine result in glyosylamine protein crosslinks and imidazolone (Scheme 2) derivatives, respectively [10].

Although glyoxal and MG have not been classified as carcinogens [11], they appear to be tumor promoters and have been shown to be direct mutagens in several cellular models [12]. In studies done with rat liver microsomal fractions (S9) or thiol compounds, the mutagenic activity of these compounds was abolished [13,14]. Therefore, the mutagenic expression of these compounds may be ameliorated by cellular antioxidant and metabolic capacity.

MG and glyoxal are detoxified endogenously primarily by the glyoxalase system, which converts glyoxal to glycolate in the presence of glutathione (GSH) [15]. Another minor detoxification pathway for glyoxal is catalyzed by oxido-reductases. The family of oxido-reductases involves the enzymes usually represented as ALR1, ALR2 and ALR3, and are called aldehyde reductase, aldose reductase and carbonyl reductase, respectively. These enzymes have a broad substrate specificity, are located in the cytosol and require NADPH or NADH to a lesser degree as a co-factor [16]. Under conditions of oxidative stress, the level of GSH decreases and this impairs glyoxal and MG detoxification by the glyoxalase system [15]. On the other hand thiamin deficiency affects the metabolism of these species via the oxido-reductase pathway due to a decrease in NADPH levels that eventually causes a decrease in GSH levels [17]. Therefore, it appears that oxidative stress and thiamin deficiency have an overlapping requirement for NADPH and GSH.

Recently, we reported that thiamin deficiency in Fischer 344 rats induced the formation of aberrant crypt foci (ACF, putative precursors of colon cancer) [18] in the absence of clinical beriberi symptoms. Furthermore, thiamin deficiency in mammalian cells in vitro markedly increased hepatocyte susceptibil-

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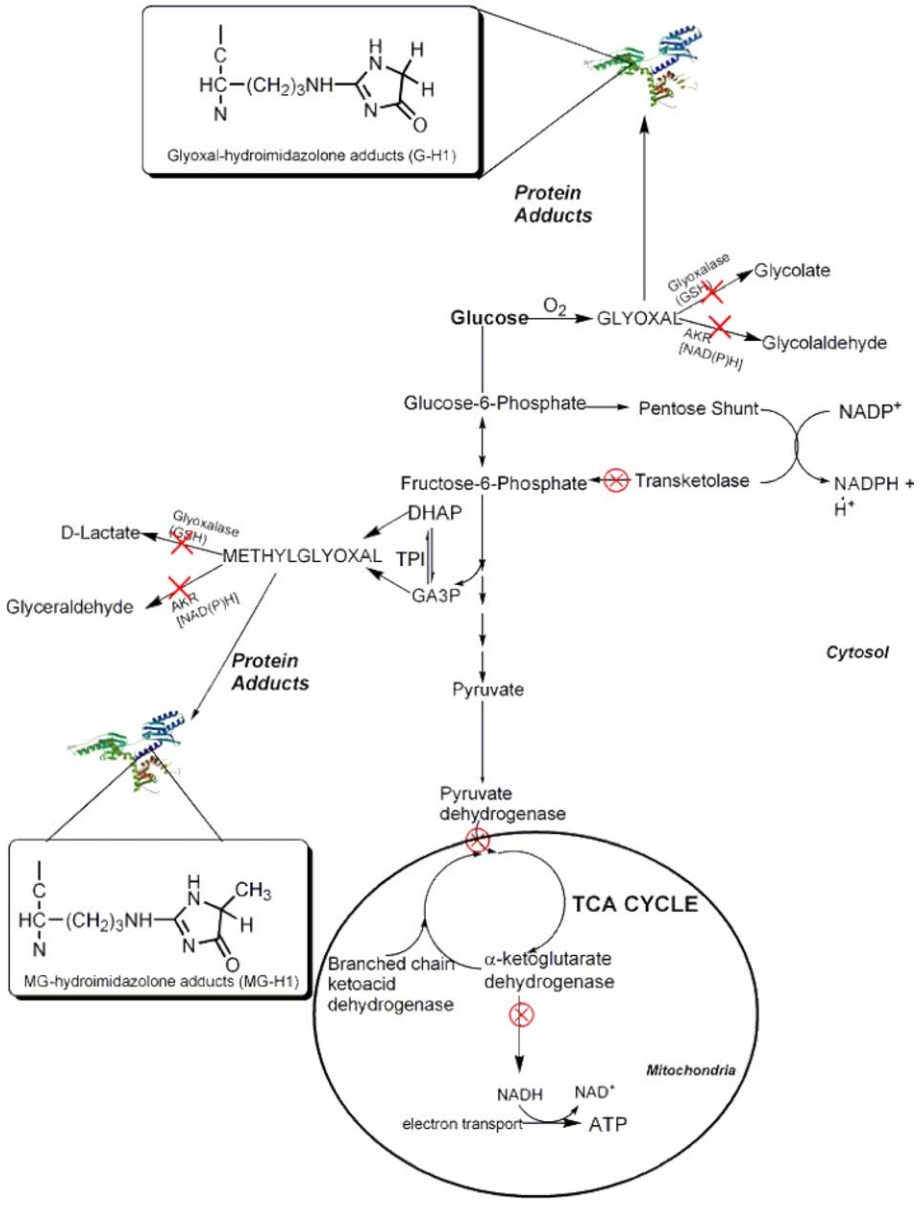

Scheme 1. Proposed mechanism of synergism between oxidative stress (i.e., MG and glyoxal) and thiamin deficiency. Thiamin deficiency causes a decrease in the activity of thiamin-dependent enzymes such as tranketolase (TK), pyruvate dehydrogenase, branched chain ketoacid dehydrogenase and α-ketoglutarate dehydrogenase (⊗). The decrease in TK activity causes a decrease in cellular NADPH levels. Under conditions of thiamin deficiency and oxidative stress the detoxification mechanisms of glyoxal and MG are decreased (×) due to lower levels of glutathione (GSH) and NADPH. The decrease in glyoxal and MG detoxification leads to an increase in their protein adducts. AKR, aldose-ketose reductase; DHAP, dihydroxyacetone phosphate; GA3P, glyceraldehyde-3-phosphate and TPI, triosephosphate isomerase.

ity to oxidative stress [2]. We hypothesize that in thiamin deficiency MG and glyoxal form macromolecular adducts, and this can result in genotoxicity in colon cells. In the presence of an oxidative stress, these effects are augmented because of decreased detoxification of these aldehydes. As a first step in testing this suggestion, we examined the effect of thiamin deficiency and oxidative stress (introduced in the form of glyoxal and MG) on hydroimidazolone adduct (Scheme 1) formation in plasma proteins.

In this study, we report that reduced dietary thiamin decreased TK activity and increased α-oxoaldehyde adduct levels. The MG adduct level was not affected by oral glyoxal or MG in the animals receiving thiamin at the control levels but was markedly increased in the animals on the thiamin-reduced diet. These observations are consistent with our suggestion that the induction of ACF with marginally thiamin-deficient diets is a consequence of the formation of MG adducts and suggest that the apparent carcinogenic effect of thiamin deficiency will be increased further in animals exposed to oxidative stress.

2. Materials and methods

Glyoxal (40% w/v), MG (40% w/v), ribose-5-phosphate, 1,2-diaminobenzene, perchloric acid ($HClO_4$) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Animal diets were prepared by Research Diets Inc. (New Brunswick, NJ).