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Hepatocyte susceptibility to glyoxal is dependent on cell thiamin content

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Abstract

Glyoxal, a reactive dicarbonyl, is detoxified primarily by the glyoxalase system utilizing glutathione (GSH) and by the aldo-keto reductase enzymes which utilizes NAD[P]H as the co-factor. Thiamin (Vitamin B_1) is an essential coenzyme for transketolase (TK) that is part of the pentose phosphate pathway which helps maintain cellular NADPH levels. NADPH plays an intracellular role in regenerating glutathione (GSH) from oxidized GSH (GSSG), thereby increasing the antioxidant defenses of the cell. In this study we have focused on the prevention of glyoxal toxicity by supplementation with thiamin (3 mM). Thiamin was cytoprotective and restored NADPH levels, glyoxal detoxification and mitochondrial membrane potential. Hepatocyte reactive oxygen species (ROS) formation, lipid peroxidation and GSH oxidation were decreased. Furthermore, hepatocytes were made thiamin deficient with oxythiamin (3 mM) as measured by the decreased hepatocyte TK activity. Under thiamin deficient conditions a non-toxic dose of glyoxal (2 mM) became cytotoxic and glyoxal metabolism decreased; while ROS formation, lipid peroxidation and GSH oxidation was increased.

Keywords: Thiamin; Glyoxal metabolism; Reactive oxygen species; Transketolase; Cytotoxicity; Advanced glycation end-products

1. Introduction

Glyoxal and its derivative, methylglyoxal (MG), are reactive α-oxoaldehydes that originate endogenously from pathways that have been linked to various pathologies such as glucose autoxidation, DNA oxidation, lipid peroxidation and thiamin deficiency [1–5]. Glyoxal and MG (glyoxals) react non-enzymatically with amino and thiol groups of biomolecules by the Maillard reaction [6,7]. The resulting Schiff base undergoes rearrangement to form relatively stable ketoamines known as Amadori products. The glycated biomolecules then undergo progressive dehydration, cyclization, and oxidation reactions to form advanced glycation end-products (AGEs) [8].

Glyoxal reacts with arginine residues leading to imidazolium formation while it reacts with lysine to form an AGE oxidative adduct N_ε-carboxymethyllysine (CML). As glyoxal is a dicarbonyl it can react with two lysine residues to form protein glycinamide crosslinks (i.e., the glyoxal-lysine dimer (GOLD)) [9,10]. Glyoxal and MG react non-enzymatically with guanyl nucleotides of DNA/RNA to form 6,7-dihydro-6,7-dihydroxy-

Abbreviations: TK, transketolase; GSH, glutathione; GSSG, oxidized glutathione; AGEs, advanced glycation end-products; MG, methylglyoxal; TPP, thiamin pyrophosphate

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imidazo[2,3-*b*]purine-9(8)one derivatives and induce mutations [11].

Protein adduct formation by glyoxal and MG results in the inactivation of critical cellular proteins which can potentially lead to apoptosis, necrosis or cell growth arrest [12]. The extent of protein glycation under physiological conditions is typically 0.01–1% of lysine and arginine residues [13] while 0.1–16% of basic phospholipids and 1 in 10^7 nucleotides in DNA are glycated [14,10]. Biomolecule glycation is suppressed under physiological conditions by enzymatic detoxification of these reactive dialdehydes. Glyoxal and MG are detoxified endogenously by two different enzymatic pathways. The primary pathway of glyoxal detoxification is the glyoxalase pathway, which is a glutathione (GSH) dependent pathway that converts glyoxal to glycolate while the aldo-keto reductase (AKR) pathway is a NAD[P]H dependent pathway that converts glyoxal to glycolaldehyde [13,15]. The accumulation of AGEs in proteins are generally regarded as contributing factors to various diseases such as cardiovascular disease, cataractogenesis, muscular disease, complications associated with diabetes mellitus, Alzheimer's disease and Parkinson's disease [8,10].

The levels of glyoxal and MG have been shown to be elevated under diabetic and thiamin (Vitamin B_1) deficient conditions. Diets deficient in thiamin have been associated with an increased presence of MG in the body fluids [16]. We have shown that thiamin deficiency may increase glyoxal and MG levels and initiate or promote the neoplastic process. Groups of male F344 rats were fed a sucrose-based diet containing 6 mg/kg (control), 1 or 0.5 mg/kg thiamine for 160 days. This resulted in increased aberrant crypt foci (ACF)/colon (a marker for colon carcinogenesis [17]) in the absence of clinical beriberi [18]. More recently, we have shown that under conditions of oxidative stress (administration of MG or glyoxal in drinking water) and dietary thiamin deficiency, plasma levels of glyoxal/MG and their protein hydroimidazolone adducts were significantly increased in F344 rats fed a sucrose-based diet [19].

It has also been found that when red blood cells were incubated with 50 mM glucose for 2 h they accumulated MG and triose phosphates. This accumulation was prevented by thiamin supplementation [4]. Furthermore, high-dose thiamin therapy prevented nephropathy [20], dyslipidemia [21] and advanced glycation of plasma proteins in streptozotocin-induced diabetic rats [22]. Benfotiamine (a lipophilic derivative of thiamin) inhibited three major biochemical pathways implicated in the pathogenesis of diabetes and prevented diabetic retinopathy in rats [23].

Thiamin in the cell is mostly metabolized by phosphorylation to its diphosphate/pyrophosphate (TPP) form catalysed by thiamin kinase. TPP is a co-factor for enzymes such as transketolase (TK), pyruvate dehydrogenase and α-ketoglutarate dehydrogenase [24]. These enzymes are involved in the maintenance of NADPH levels and carbohydrate metabolism. Under thiamin deficient conditions, the metabolism of glyoxal and MG by the aldo-keto reductase pathway is affected as the NADPH supply of the cell is decreased. NADPH is also an important co-factor for antioxidant defense enzymes such as glutathione reductase (GR). GR is required to convert oxidized glutathione (GSSG) to reduced GSH. Decrease in GR activity leads to a decrease in GSH levels of the cell and a decrease in the primary pathway of glyoxal and MG metabolism by the glyoxalase pathway. As glyoxal metabolism is dependent on cellular GSH and NADPH levels and thiamin helps maintain cellular levels of these species, then supplementation with thiamin should prevent glyoxal-induced toxicity in isolated rat hepatocytes by increasing its metabolism.

In the present study, we have investigated the possible protective role of thiamin against glyoxal toxicity in isolated rat hepatocytes. Supplementation with thiamin (3 mM) resulted in cytoprotection and restored NADPH levels, glyoxal detoxification and mitochondrial membrane potential. Hepatocyte reactive oxygen species (ROS) formation, lipid peroxidation and GSH oxidation were also decreased. Furthermore, under thiamin deficient conditions a non-toxic dose of glyoxal (2 mM) was cytotoxic and glyoxal metabolism decreased; while ROS formation, lipid peroxidation and GSH oxidation increased.

2. Materials and methods

2.1. Materials

1-Bromoheptane, MG (40%, w/v), glyoxal (40%, w/v), 2,4-dinitrofluorobenzene (DNFB), 2,7-dichlorofluorescin diacetate (DCFH-DA), thiamin, oxythiamin, rhodamine 123, protein A-Sepharose CL-4B matrix were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Type II Collagenase was purchased from Worthington (New Jersey, USA). Polyclonal 4-hydroxynonenal (4-HNE) antibody was purchased from Alpha Diagnostics, International Inc. (San Antonio, TX). Enhanced chemiluminescence