Toxicity of glyoxals – role of oxidative stress, metabolic detoxification and thiamine deficiency

N. Shangari*, W.R. Bruce†, R. Poon‡ and P.J. O’Brien*1

*Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Toronto, 19 Russell St., Toronto, ON, Canada M5S 2S2, †Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, FitzGerald Building, 150 College Street, Toronto, ON, Canada M5S 3E2, and ‡Bureau of Chemical Hazards, Health Canada, Ottawa, ON, Canada

Abstract

Glyoxals are reactive α-oxoaldehydes that are formed endogenously from sugars, the levels of which are increased in various pathological conditions associated with hyperglycaemia and thiamine deficiency. However, the molecular cytotoxic mechanisms of glyoxal are not known. Results presented here and in the other studies cited provide a glimpse into the cytotoxicity mechanisms involved and their pathological implications. We found that glyoxal (10 μM) markedly increased the susceptibility of hepatocyte glutathione (GSH) to oxidation by hydrogen peroxide (H2O2) and markedly increased cytotoxicity by compromising the cellular antioxidant enzyme system. At higher concentrations, glyoxal was cytotoxic towards hepatocytes, which can be attributed to GSH depletion, oxidative stress and mitochondrial toxicity. Aminoguanidine or penicillamine protected the hepatocytes. Glyoxal cytotoxicity was prevented by increasing glyoxal metabolism with thiamine or NAD(P)H generators, and was increased in GSH- or thiamine-deficient hepatocytes. It was also found that feeding rats reduced thiamine levels in a diet high in simple sugars increased the number of aberrant crypt foci/colon in the absence of clinical evidence of beriberi. This was associated with decreased plasma thiamine and low erythrocyte transketolase activity. Western diets, which are frequently poor in thiamine and high in sugars, could result in increased levels of endogenous glyoxals, which in turn may lead to a predisposition to AGE (advanced glycation end-product)-related pathologies and neoplastic conditions.

Introduction

Glyoxals [glyoxal and MG (methylglyoxal)] are reactive α-oxoaldehydes that originate endogenously from sugars, the levels of which have been linked to various pathologies, such as glucose autoxidation, DNA oxidation, lipid peroxidation and beriberi (thiamine deficiency) [1–5]. Glyoxals also constitute a significant portion of air-borne carbonyl compounds originating from automotive exhausts [6].

Glyoxals react with proteins by forming covalent bonds with the free amino groups of biomolecules by a non-enzymatic reaction called glycation [2,7]. The resulting Schiff base then undergoes rearrangement to form relatively stable ketoamines known as Amadori products. The glycated biomolecules then undergo progressive dehydration, cyclization, oxidation and rearrangement to form AGEs (advanced glycation end-products) [8]. Glyoxal reacts with arginine leading to imidazolium formation, while it reacts with lysine to form an AGE oxidative adduct, N-(carboxymethyl)lysine. As glyoxal is a dicarbonyl, it can react with two lysine residues to form protein glycinamide cross-links (i.e. the glyoxal–lysine dimer) [1,5]. Glyoxal and MG react non-enzymatically with guanyl nucleotides of DNA/RNA to form 6,7-dihydro-6,7-dihydroxy- imidazo[2,3-b]purine-9(8)one derivatives and induce mutations [9–13].

Although glyoxal and MG have not been classified as carcinogens [14], they appear to be tumour promoters and have been shown to be direct mutagens in several cellular models [15]. Interestingly, their mutagenic activity was abolished by a rat liver microsomal fraction (S9) or by thiol compounds [16,17]. When human lymphocytes were tested, MG mutagenicity was also decreased by the presence of S9 [18]. The mutagenic expression of glyoxals may therefore be ameliorated by cellular antioxidant and metabolic capacity.

The extent of protein glycation under physiological conditions is typically 0.01–0.01% of lysine and arginine residues [19], while 0.1–16% of basic phospholipids and 1 in 107 nucleotides in DNA are glycated [20]. Biomolecule glycation is suppressed under physiological conditions by enzymatic detoxification of α-oxoaldehydes. Glyoxal and MG are detoxified by the cytosolic glyoxalase I and glyoxalase II system to form glycolate and D-lactate, respectively. The rate of detoxification of glyoxal and MG by the cell is dependent on the cytosolic GSH concentration [21].

Hyperglycaemia or abnormal glucose metabolism leads to an increase in glyoxal and MG levels in the blood [22]. It now appears likely that the elevated levels of glyoxals result from the inhibition of thiamine-dependent enzymes in the pentose phosphate pathway and citric acid cycle, leading to
a decrease in NADPH formation by glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase respectively [4,23].

**Oxidative stress and glyoxal**

Under conditions of oxidative stress, the subsequent decrease in cellular GSH concentration impairs glyoxal and MG detoxification [19]. Glyoxal, probably through the process of protein glycation (e.g. arginine), inactivates glutathione reductase, glutathione peroxidase, enzymes that supply NADPH and superoxide dismutase ([24,25]; N. Shangari and P.J. O'Brien, unpublished work). 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 [19,27–30]. Whereas glycated nucleic acids are subject to repair by highly efficient excision/insertion mechanisms, the damaged glycated proteins need to be proteolytically degraded by highly regulated protease complexes. Replacement proteins then have to be synthesized and relocated within the cell [31]. The accumulation of AGEs in proteins under these conditions is generally regarded as a contributory factor to various diseases such as cardiovascular disease, cataractogenesis, muscular disease, complications associated with diabetes mellitus, Alzheimer’s disease and Parkinson’s disease [3,27].

We found that low concentrations of glyoxal (<10 μM) markedly increased rat hepatocyte cytotoxicity and oxidation of GSH induced by H₂O₂. These results suggest that glyoxal readily compromised the cellular enzymic antioxidant system by inhibiting glutathione reductase and/or enzymes supplying NADPH. However, at much higher concentrations, glyoxal by itself was cytotoxic, depleted GSH, produced ROS (reactive oxygen species) and collapsed the mitochondrial membrane potential. Glyoxal also induced, probably through lipid peroxidation, the formation of formaldehyde, a carcinogenic and mutagenic aldehyde [32]. Antioxidants or ROS scavengers prevented glyoxal-induced mitochondrial toxicity and cytotoxicity. Glycolytic substrates (e.g. fructose) and NADH generators (e.g. sorbitol) also inhibited glyoxal-induced cytotoxicity and prevented the decrease in mitochondrial membrane potential, suggesting that mitochondrial toxicity contributed to the cytotoxic mechanism. A molecular cytotoxic mechanism is shown in Scheme 1. Glyoxal cytotoxicity was also prevented by the glyoxal traps D-penicillamine, aminoguanidine or pyridoxamine, which were still cytoprotective when added 20 min after glyoxal (N. Shangari and P.J. O’Brien, unpublished work).

**Thiamine deficiency, glyoxal formation and toxicity**

Thiamine in its diphosphate form (TDP) is an important coenzyme for transketolase, pyruvate dehydrogenase, α-oxoglutarate dehydrogenase and the branched-chain α-oxoacid
dehydrogenase complex, enzymes that are involved in the pentose phosphate pathway and citric acid cycle energy production (Scheme 2) [33]. Thiamine also increased erythrocyte transketolase activity and decreased MG accumulation when erythrocytes were incubated with 50 mM glucose [4].

In order to determine whether decreases in the availability of thiamine affect glyoxal levels, we made isolated rat hepatocytes thiamine-deficient by pre-incubation with oxythiamine. Glyoxal metabolism was inhibited in thiamine-deficient hepatocytes, and these hepatocytes were also more susceptible to glyoxal cytotoxicity, whereas hepatocytes pre-incubated with thiamine metabolized glyoxal more readily and were more resistant. Furthermore, to test the possibility that thiamine deficiency may increase glyoxal levels and might initiate or promote the neoplastic process, groups of male F344 rats were fed a sucrose-based diet containing 6 (control), 1 or 0.5 mg/kg thiamine for 160 days. This resulted in an increased number of aberrant crypt foci/colon (a marker for colon carcinogenesis [34]) in the absence of clinical evidence of beriberi, and markedly decreased plasma thiamine levels and erythrocyte transketolase activity (W.R. Bruce, R. Furrer, N. Shangari, P.J. O’Brien, A. Medline and Y.P. Wang, unpublished work).

The above described experimental results and brief literature review provides a glimpse into the relationship between elevated glyoxal and its pathological expression such as cytotoxicity, diabetic complications and aberrant crypt foci of the colon, and the possible role of oxidative stress, metabolic detoxification and thiamine deficiency in modulating cellular susceptibility to glyoxals. It may be hypothesized that elevated levels of glyoxals associated with pathological conditions or exogenous exposure may enhance AGE-related adverse effects. Western diets that are frequently high in sugar and marginal in thiamine may create nutritional conditions that produce high endogenous glyoxals, and hence increase the risk of pre-neoplastic or neoplastic conditions in the colon.

We thank our sponsors Cancer Research Society with CIHR (Canadian Institute of Health Research) and Health Canada Contract for their support of this research. Also we thank Rudolf Furrer, Alan Medline and Yanping Wang for their help.
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Received 2 July 2003