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Mini review

Dicarbonyl stress in cell and tissue dysfunction contributing to ageing and disease



Naila Rabbani, Paul J. Thornalley*

Clinical Sciences Research Laboratories, Warwick Medical School, University of Warwick, University Hospital, Coventry CV2 2DX, UK

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ABSTRACT

Dicarbonyl stress is the abnormal accumulation of dicarbonyl metabolites leading to increased protein and DNA modification contributing to cell and tissue dysfunction in ageing and disease. Enzymes metabolising dicarbonyls, glyoxalase 1 and aldoketo reductases, provide an efficient and stress-response enzyme defence against dicarbonyl stress. Dicarbonyl stress is produced by increased formation and/or decreased metabolism of dicarbonyl metabolites, and by exposure to exogenous dicarbonyls. It contributes to ageing, disease and activity of cytotoxic chemotherapeutic agents.

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1. Dicarbonyl stress – a definition

Dicarbonyl stress is the abnormal accumulation of α -oxoaldehyde metabolites leading to increased modification of protein and DNA contributing to cell and tissue dysfunction in ageing and disease [1]. Examples are the increased methylglyoxal (MG) in ageing plants [2], increased MG-protein modification in ageing human lens [3], increased plasma and tissue concentration of MG in diabetes [4], and increased concentrations of MG, glyoxal, 3-deoxyglucosone (3-DG) and other dicarbonyls in renal failure [5]. Dicarbonyl stress is caused by an imbalance of the formation and metabolism of dicarbonyl metabolites and also by increased exposure to exogenous dicarbonyls. Typical concentrations of glyoxal, MG and 3-DG are 50–150 nM in human plasma and 1–4 μ M in plant and mammalian cells [2,5,6]. When dicarbonyl concentrations increase beyond this there is potential for protein and cell dysfunction leading to impaired health and disease.

2. Formation and metabolism of dicarbonyls

Sources of formation of dicarbonyl metabolites, glyoxal, MG and 3-DG, and routes of their metabolism are summarised in Table 1. MG is formed at relatively high flux mainly by the trace level,

0.05–0.1 % flux, degradation of triosephosphates, glyceraldehyde-3-phosphate (GA3P) and dihydroxyacetonephosphate (DHAP). This increases with increased glucose metabolism, inhibition of GA3P dehydrogenase and impaired disposal of GA3P by the reductive pentosephosphate pathway. It may also arise from other metabolic pathways where triosephosphates are intermediates: gluconeogenesis, glyceroneogenesis and photosynthesis. Dicarbonyls in foodstuffs are completely or partly metabolised and/or react with proteins before absorption in the gastrointestinal tract and impose dicarbonyl stress mainly in the gastrointestinal lumen [7,8]. Glyoxal and MG are metabolised mainly by glyoxalase 1 (Glo1) of the glutathione (GSH)-dependent glyoxalase system, with minor metabolism by aldoketo reductases (AKRs) and aldehyde dehydrogenases (ADHs). 3-DG is metabolised to 3-deoxyfructose by AKRs and to 3-deoxy-2-ketogluconate by ADH – Table 1 and Fig. 1. Glo1, AKRs and ADH are under stress-responsive control by transcription factor Nrf2 through regulatory antioxidant response elements (AREs). Nrf2 activation in dicarbonyl stress may involve reversible binding of dicarbonyls to reactive cysteine residues in regulatory inhibitory protein Keap1.

Other proteins, “glyoxalase III” and DJ1, were proposed as glyoxalases but their low catalytic efficiency suggests this is unlikely [9]. DJ1 was also proposed as a catalyst for de-glycating early-stage reversible reactions of MG with cysteine, lysine and arginine residues [10]. Comparison with *in situ* kinetics [11], however, suggests this does not compete effectively with the spontaneous reversal and metabolism of by Glo1.

* Corresponding author.

E-mail address: P.J.Thornalley@warwick.ac.uk (P.J. Thornalley).

Table 1
Formation and metabolism of dicarbonyl metabolites and adducts formed in protein and DNA.

Dicarbonyl metabolite	Formation	Metabolism	Major protein and DNA adducts
Glyoxal	<ol style="list-style-type: none"> 1. Lipid peroxidation. 2. Degradation of glycated proteins. 3. Oxidative degradation of serine (via glycolaldehyde). 4. Monosaccharide degradation. 5. Degradation of nucleotides. 6. Food and beverages. 	<ol style="list-style-type: none"> 1. Glyoxalase 1 (MAJOR). 2. Aldoketo reductases 1B1 (aldose reductase), 1B3 and 1B8 (MINOR – except in renal medulla). 	Protein: N _ω -carboxymethylarginine (CMA), hydroimidazolone (G-H1) and N _ε -carboxymethyl-lysine (CML). DNA: imidazopurinone GdG and N ₂ -carboxymethyl-deoxyguanosine (CMDG).
MG	<ol style="list-style-type: none"> 1. Degradation of GA3P and DHAP in anaerobic glycolysis, gluconeogenesis, glyceroneogenesis and photosynthesis (MAJOR). 2. Ketone body metabolism (MINOR – expect in ketosis). 3. Threonine catabolism (MINOR). 4. Degradation of glycated protein (MINOR). 5. Monosaccharide degradation. 6. Food and beverages (MINOR). 	<ol style="list-style-type: none"> 1. Glyoxalase 1 (MAJOR). 2. Aldoketo reductases 1A4, 1B1 (aldose reductase) and 1B3; “MG reductase” (MINOR – except in renal medulla). 3. Aldehyde dehydrogenase E1, E2 and E3; “MG dehydrogenase” (MINOR). 	Protein: hydroimidazolone (MG-H1), and N _ε (1-carboxyethyl)lysine (CEL). DNA: imidazopurinone MdG and N ₂ -(1-carboxyethyl)deoxyguanosine (CEdG).
3-DG	<ol style="list-style-type: none"> 1. Enzymatic repair of glycated proteins (MAJOR). 2. Degradation of glycated protein (MINOR). 3. Monosaccharide degradation (MINOR). 4. Metabolism of fructose (MINOR). 5. Food and drink (IMPORTANCE VARIES). 	Aldoketo reductases 1A4, 1B1 and 1B3; “3-DG reductase activity” (MAJOR). Aldehyde dehydrogenase 1A1; “3-DG dehydrogenase” (MINOR).	Protein: hydroimidazolone isomers 3DG-H and pyrraline (latter mostly from food). DNA: unknown.

Relative importance of pathways of formation and metabolism of dicarbonyls is indicated where known.

3. Biochemical consequences of dicarbonyl stress

Dicarbonyl stress produces increased rate of reaction of dicarbonyls with protein, nucleotides and basic phospholipids. The process is dicarbonyl glycation and the adducts formed are advanced glycation endproducts (AGEs).

Reaction with proteins is directed to arginine residues forming dihydroxyimidazolidine and hydroimidazolone adducts. The hydroimidazolone derived from MG, MG-H1, is one of the most quantitatively and functionally important AGEs in physiological systems. There are also minor lysine-derived AGEs formed: N_ε-carboxymethyl-lysine (CML), N_ε(1-carboxyethyl)lysine (CEL) and pyrraline formed from glyoxal, MG and 3-DG respectively – Fig. 2. The major source of CML formation, however, is the oxidative degradation of N_ε-fructosyl-lysine residues. Pyrraline is formed exclusively at high temperatures and hence is a marker of exposure to AGEs from food.

Dicarbonyl glycation is particularly insidious as it is directed to arginine – the amino acid residue with highest probability of location in functional sites of proteins, modification induces loss of charge of the side chain guanidino group and functionally important arginine residues tend to be those most reactive towards dicarbonyl glycation. The extent of glycation of proteins by dicarbonyls is low, usually 1–5%, but may increase in ageing and disease. Proteins modified by glyoxal and MG in dicarbonyl stress are recognised as mis-folded and directed to the proteasome for proteolysis. In yeast an unfocused gene deletion analysis showed strains deleted for genes of ubiquitin-dependent protein degradation were sensitive to glyoxal and MG toxicity [12].

Dicarbonyl glycation of cellular and extracellular matrix (ECM) proteins mediates: mitochondrial protein dysfunction and increased formation of reactive oxygen species (ROS) [13], inflammatory protein expression (receptor for advanced glycation end-product RAGE, S100 proteins and HMGB1) [14], mitochondrial pathway activated apoptosis [15] and cell detachment from the extracellular matrix and anoikis [16]. The dicarbonyl proteome, proteins susceptible to dicarbonyl modification, is under investigation. In pilot studies, we identified 344 of 1366 proteins modified with MG in cytosolic protein extracts of human endothelial cells with MG-H1 content increased 10-fold from control levels and 12 of 1027 proteins in control samples [17].

Glyoxal and MG are important precursors of DNA adducts in physiological systems: major adducts are imidazopurinones GdG and MGdG – nucleotide AGEs. Increased nucleotide AGEs was associated with DNA strand breaks and mutagenesis [18].

4. Physiological consequences of dicarbonyl stress

Where dicarbonyl stress occurs there is potential for increased cell anoikis and apoptosis and increased dysfunction, turnover and depletion or compensatory increase expression of the dicarbonyl proteome. MG permeates cell plasma membranes by passive diffusion of the unhydrated form. This is rate limited by MG dehydration, giving a half-life of ~4 min [2]. The half-life for metabolism of MG by the glyoxalase system to D-lactate for metabolism of MG by the glyoxalase system to D-lactate in *in situ* rates of D-lactate formation in cells is *ca.* 10 min with free MG mostly (>95%) reversibly bound to protein. The rate of irreversible binding to protein in plasma was *ca.* 3.6 h. This implies that part of the MG formed in cells leaks out from the site of formation and may diffuse through interstitial fluid into plasma and thereafter permeate back into interstitial fluid and cells of other tissues. Also, MG formed from the degradation of glycated proteins in the extracellular compartment enter may enter cells for metabolism by Glo1 and AKRs. The locus of dicarbonyl stress and related pathogenesis linked to MG accumulation is therefore likely sensitive to local decrease of Glo1 expression and activity – Fig. 3.

GLO1 is a hotspot for copy number variation in human and mouse genomes, giving rise to a 2–4-fold increase in Glo1 expression but is only found at 2–3% prevalence [19]. Deletion of GLO1 is embryonically lethal in mice and human subjects. GLO1 contains regulatory elements: metal response element, insulin response element, E2F4, AP-2 α and ARE elements, as reviewed [9]. It is negatively regulated by HIF1 α in hypoxia [20] and also by RAGE [9]. Hypoxia may be an important physiological driver of dicarbonyl stress as it both increases MG formation by flux through anaerobic glycolysis and likely decreases Glo1 expression.

5. Comparison and interactions with oxidative stress

Dicarbonyl stress may be both a cause and consequence of oxidative stress. Overexpression of Glo1 in *Caenorhabditis elegans* decreased MG-H1 content of mitochondrial proteins and thereby

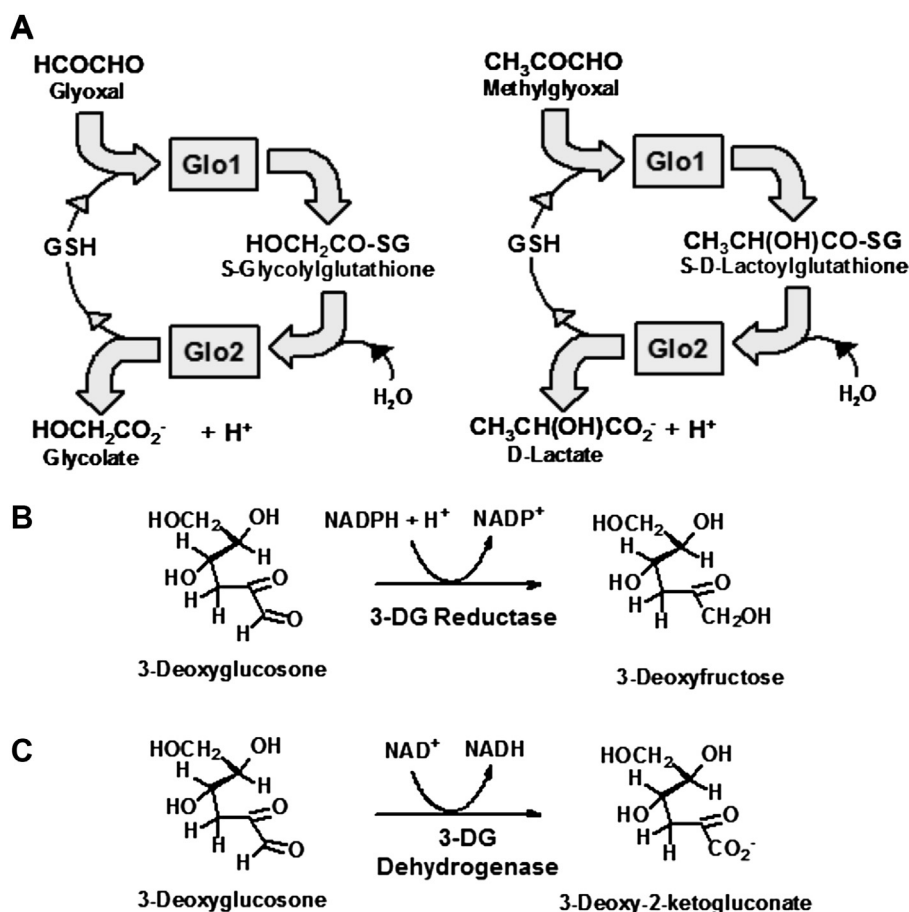


Fig. 1. Metabolism of dicarbonyls – the enzymatic defence against glycation. A. Metabolism of glyoxal and methylglyoxal by the glyoxalase system. B. and C. Metabolism by 3-DG reductase and dehydrogenase.

formation of ROS [13]. Similarly overexpression of Glo1 in human aortal endothelial cells decreased formation of ROS in high glucose concentration [14]. Oxidative stress may also lead to the accumulation of triosephosphates and thereby increase the formation of MG which occurs non-oxidatively [21]. Also, decrease of cellular GSH decreases *in situ* activity of Glo1 and thereby the metabolism of glyoxal and MG [22], and decrease of cellular NADPH decreases the *in situ* activity of aldoketo reductases and thereby the metabolism of 3-DG.

The locus of reactivity of ROS in physiological systems is related to the diffusion distance before reaction with substrates, as defined [23]. Similar considerations of the irreversible reactions of MG indicates the diffusion distance is *ca.* 2–3 cm, suggesting that MG has relatively long range and half-life to identify and modify sensitive sites of proteins, often leading to protein inactivation and dysfunction.

6. Dicarbonyl stress in ageing and disease

6.1. Ageing

The link of dicarbonyl stress to ageing was established in a functional genomics study of Glo1 in nematode *C. elegans* [13]. MG-derived AGEs increased in human lens with age and was linked to cataract formation [3]. Decreased Glo1 activity was associated with impaired wound healing [24]. Dicarbonyl stress is also likely involved in

senescence of plants. MG-H1 was a major AGE in *Arabidopsis* leaves [25] and dicarbonyl content of broccoli increased with age [2].

6.2. Obesity

In obesity there was genetic linkage of GLO1 to body weight in mice [26] and to upper-arm circumference and supra-iliac skinfold thickness in human subjects [27]. Latest studies suggest that Glo1 is decreased in white adipose tissue in mice on a high fat diet and overexpression of Glo1 suppresses gain in body weight and adiposity with similar food consumption as wild-type control [28]. Dicarbonyl stress may be a mediator of obesity and insulin resistance.

6.3. Diabetes and diabetic vascular complications

Formation of MG is increased in cells with GLUT1 glucose transport incubated in high glucose concentration. Glo1 activity is decreased in the kidney, retina and peripheral nerve in experimental diabetes – which may occur via inflammatory signalling via RAGE and hypoxia [29,30]. These features synergise to increase MG concentration. Plasma MG is increased by up to 5–6 fold in patients with diabetes [4]. Functional genomics studies with Glo1 deficient and Glo1 overexpressing transgenic mice support increased MG as a factor linked to the development of diabetic microvascular complications (nephropathy, retinopathy and neuropathy) [29,31,32].

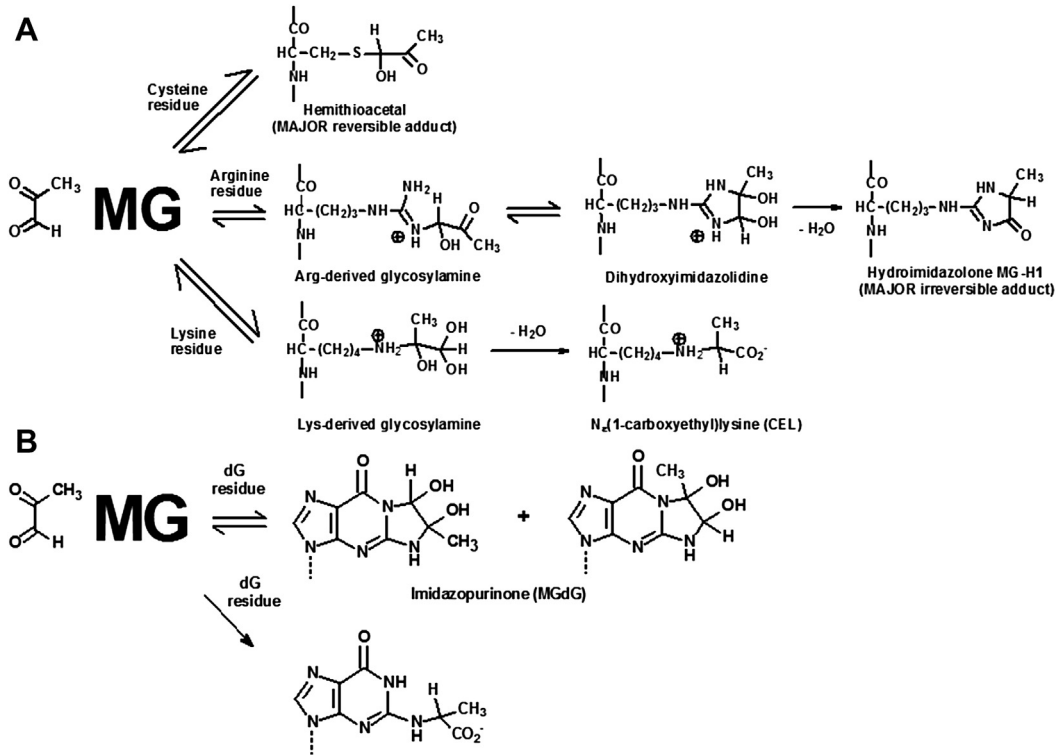


Fig. 2. Biochemistry of dicarbonyl glycation – glycation by methylglyoxal. A. Adduct residues formed in proteins. Other minor AGEs: argpyrimidine and crosslinks MOLD and MODIC. B. Nucleotide AGEs [17].

6.4. Chronic renal disease

Dicarbonyl stress came to prominence in renal disease in relation to exposure of patients with end stage renal disease (ESRD)

receiving peritoneal dialysis (PD) being exposed to glyoxal, MG and 3-DG in dialysis fluids. Further studies showed patients with ESRD on haemodialysis also had increased plasma MG and flux of formation of dicarbonyl-derived AGEs [5,33]. The cause of dicarbonyl

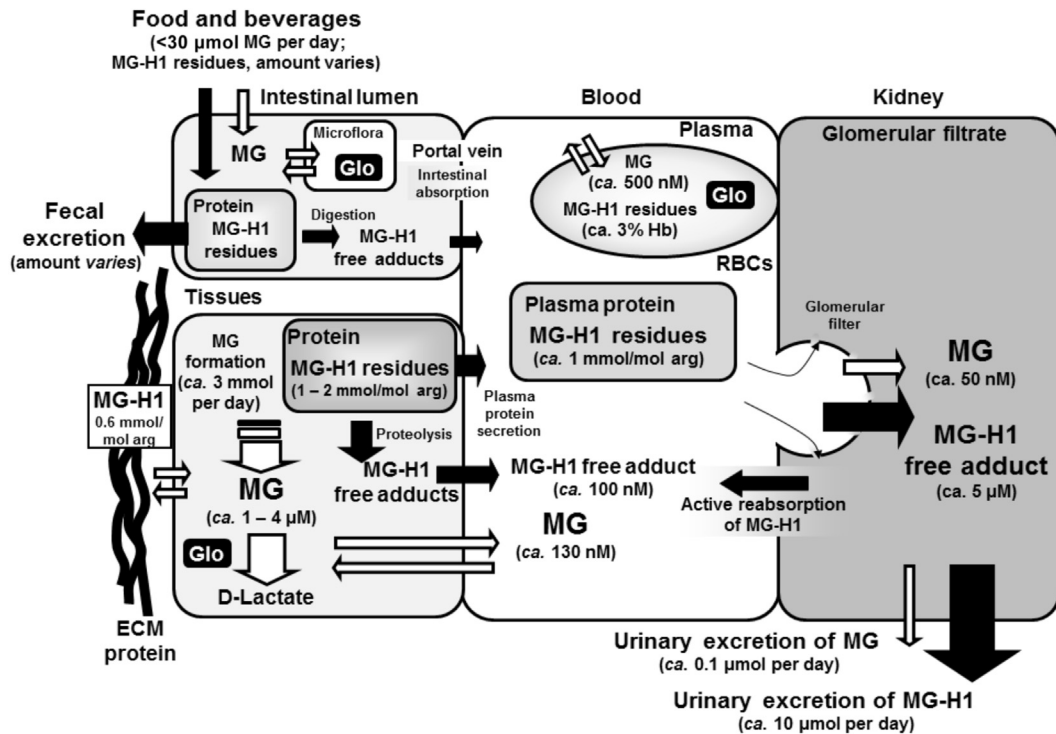


Fig. 3. Molecular physiology of dicarbonyl stress. Biodistribution of MG and the MG-H1 in normal human physiological function. MG-H1 is shown as residues of protein and free adducts elsewhere – the latter formed mainly by cellular proteolysis (or digestion for ingested food proteins). Key: open and solid arrows – flows of MG and MG-H1 respectively; Glo – metabolism of MG by the glyoxalase system. Key: Hb, haemoglobin; RBC, red blood cell.

stress remains unclear. It is unlikely due to decreased dicarbonyl excretion as there is little in normal health [7], although it is linked to renal function as dicarbonyl stress was a developing feature of both experimental nephrectomy and ureteral ligation [34]. Decreased Glo1 expression by hypoxia and inflammation, hypoxia-induced increased anaerobic glycolysis and decreased disposal of triosephosphates by the reductive pentosephosphate pathway (enzymes of which are inhibited by uraemic toxins) leading to increased formation of MG are likely causes. Ageing-related decline in renal function and interstitial thickening was prevented in transgenic rats overexpressing Glo1 [35].

6.5. Cardiovascular disease (CVD)

Dicarbonyl stress in plasma likely contributes to CVD risk through induction of dyslipidaemia. MG modification of LDL induced atherogenic transformation to small, dense LDL with increased affinity for arterial walls through binding to heparan sulphate proteoglycans [36]. MG modification of HDL induced restructuring of the HDL particles, increasing density, decreasing stability and plasma half-life *in vivo* [37]. Chemical inhibition of Glo1-induced atherosclerosis in apoE deficient mice [38]. A recent clinical integrative genomics study of >90,000 CVD cases and controls revealed Glo1 is a driver of CVD [39].

6.6. Carcinogenesis, tumour growth and cancer chemotherapy

Recent studies suggest a duality of functions of Glo1 in carcinogenesis and tumour growth. In a p53 knockout, Myc over-expression model of liver carcinogenesis, a genome-wide scan found Glo1 is a tumour suppressor protein [40]. Conversely, over-expression of Glo1 in tumours may be permissive for growth with high glycolytic activity and high flux of MG formation [18]. Increased expression of Glo1 in tumours is due to GLO1 amplification in some cases – particularly breast cancer and lung cancer [41], and may also be linked to mutation and increased transcriptional activity of Nrf2 through ARE-linked upregulation of Glo1 transcription [42]. It also confers multidrug resistance but sensitivity to siRNA silencing and chemical inhibition of Glo1 [41].

6.7. Neurological disorders

A rare clinical Glo1 deficiency was linked to high risk of severe schizophrenia [43]. Experimental deficiency of synuclein- α increased dicarbonyl content and Glo1 expression in brain stem, midbrain and cortex – suggesting that synuclein- α may prevent dicarbonyl stress and this function may be impaired in synucleinopathies such as Parkinson's disease [44]. Increased MG-H1 free adduct was found in cerebrospinal fluid of patients with Alzheimer's disease [45] and Glo1 expression was increased in early-stage and decreased in late-stage disease [46], suggesting that dicarbonyl stress may be a feature of Alzheimer's disease. There was also a link of GLO1 duplication to anxiety-like behaviour in mice which may rather be due to a proximate genetic locus co-duplicated with Glo1 [19].

6.8. Malaria

Cell permeable Glo1 inhibitor BBGD had potent anti-malarial activity against the red blood cell stage of *Plasmodium falciparum*. This stage of the malarial parasite growth cycle has only anaerobic glycolysis with an associated high flux of MG formation [47].

7. Dicarbonyl stress-based therapeutics

Dicarbonyl stress may be alleviated by prevention of dicarbonyl formation, scavenging of dicarbonyls and enhancing the expression of enzymes of dicarbonyl metabolism – particularly Glo1. Such interventions may be beneficial in the prevention and treatment of obesity, type 1 and type 2 diabetes and their vascular complications, renal failure and CVD, and also support healthy ageing. High dose thiamine supplements for prevention of type 2 diabetes and vascular complications of diabetes may work partly by prevention of MG formation [48,49]. Dicarbonyl scavengers showed some promise but the high reactivity required for effective scavenging produces associated toxicity and instability which prohibited development [5]. Discovery of Glo1 inducers which work through activation and binding of Nrf2 to the GLO1 functional ARE offers an alternative that is more effective and safe [50]. Cell permeable Glo1 inhibitors which are inducers of dicarbonyl stress may find use as anti-tumour and anti-microbial agents for treatment of Glo1-linked multidrug resistant tumours and microbial infections. Systems modelling of the glyoxalase pathway is beneficial in assessment of the potency of Glo1 inducer or Glo1 inhibitor required to achieve the desired pharmacological and therapeutic effects [9].

8. Technical issues for investigators entering dicarbonyl stress research

A compilation of methods for dicarbonyl and glyoxalase research can be found in proceedings of a recent conference workshop – see Ref. [1] and related papers. Commercial sources of MG typically contain major contamination and there are many potential interferences in measurement of dicarbonyls. We have described protocols to prepare high purity MG, for reliable assay of dicarbonyls and systems modelling for prediction of dicarbonyl concentrations [2,6]. For model glycated proteins prepared *in vitro* similar low extents of glycation are appropriate for physiological relevance [17].

9. Closing remarks

Exposure to dicarbonyl metabolites is an intrinsic feature of physiological systems as a corollary to the presence of triosephosphate glycolytic intermediates and other dicarbonyl precursors. Formation and enzymatic metabolism of dicarbonyls maintaining low, tolerable levels of protein and DNA modification establishes the conditions for dicarbonyl stress.

Conflict of interest

The authors declare no conflict of interest.

Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.bbrc.2015.01.140>.

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