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Inhibition of lysozyme aggregation by α-oxoaldehydes: Insight into a novel therapeutic potential of the compounds in the treatment of systemic amyloidosis

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ABSTRACT: Systemic amyloidosis is a hereditary disorder that mostly arises as a result of specific point mutations to the wild type gene of lysozyme, forming mutant lysozyme variants leading to aggregation of the protein. Targeting the aggregates of lysozyme is a potential approach to curb systemic amyloidosis with possible therapeutic implications. For this purpose, several drug molecules and small molecule ligands have been tested for their ability to disaggregate protein fibrils. In recent studies we have found that the reactive α-oxoaldehydes, glyoxal and methylglyoxal, to inhibit the aggregation of lysozyme in vitro. We discuss a novel therapeutic potential of the compounds in the treatment of hereditary systemic lysozyme amyloidosis and protein conformational disorder.

KEYWORDS: Lysozyme; Systemic amyloidosis; Glyoxal; Methylglyoxal; Advanced Glycation End products

I. INTRODUCTION

Deviations from the normal folding route forms misfolded states which serve as a precursor for aggregate formation **(Naeem & Fazili, 2011)**. Protein aggregates in an advanced stage mature into amyloid fibrils **(Fazili, Bhat, & Naeem, 2014)**. Formation of amyloid fibrils or inclusion bodies is the hallmark of a number of human pathologies especially Alzheimer's disease, Parkinson's disease, Amyotropic lateral sclerosis, cataract etc **(Fazili et al., 2014)**. Systemic amyloidosis is a non-neuropathic hereditary disorder which mainly arises as a result of specific point mutations to the wild type gene of lysozyme forming mutant lysozyme variants, affecting mainly kidney, liver and spleen **(Buell et al., 2011)**. The mutations favour the formation of misfolded conformers which in turn leads to aggregation of lysozyme **(Gazova et al., 2008)**. Targeting the fibrils of lysozyme that are actually involved in the pathogenesis of hereditary systemic lysozyme amyloidosis can serve as positive therapeutics to curb the disorder. For this purpose, several drug molecules and small molecule ligands have been tested for their ability to disaggregate protein fibrils **(Borana, Mishra, Pissurlenkar, Hosur, & Ahmad, 2014; Khan et al., 2019b; Ramazzotti et al., 2016)**.

II. NON-ENZYMATIC PROTEIN GLYCATION

Reducing sugars react with amino groups of proteins, a process known as non-enzymatic glycation (Maillard reaction) resulting in browning, fluorescence and crosslinking of proteins **(Ahmad, Ahmad, & Moinuddin, 2011)**. The reaction consists of several steps, including Schiff's base formation, Amadori rearrangement, etc., finally leading to formation of advanced glycation end products (AGEs) **(Figure 1)**. The α-dicarbonyl compounds namely, glyoxal, methylglyoxal and 3-deoxyglucosone are known to initiate Maillard reaction, and are more reactive than the parent hexose sugars with respect to their ability of protein modification and AGE formation **(Ahmed, Dobler, Dean, & Thornalley, 2005; Kalapos, 1999)**. Methylglyoxal level increases in different pathological conditions including diabetes mellitus **(McLellan, Thornalley, Benn, & Sonksen, 1994; Ramasamy, Yan, & Schmidt, 2006)** leading to formation of AGEs with different long-lived proteins, namely insulin **(Oliveira et al., 2011)**, human serum albumin **(Ahmed et al., 2005)**, α-synuclein **(Lee, Park, Paik, & Choi, 2009)**, superoxide dismutase **(Khan, Chaturvedi, et al., 2014)**, as well as Aβ peptide **(Emendato et al., 2018)**. Methylglyoxal-induced modifications of heme proteins, myoglobin and hemoglobin have been reported in recent studies **(Banerjee, Maity, & Chakraborti, 2016; Banerjee, 2017)**. It forms argpyrimidine, hydroimidazolones and tetrahydropyrimidine with arginine residues, whereas carboxyethyllysine and methylglyoxal-

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lysine dimer are the AGEs formed with lysine residues. The structures of some of the methylglyoxal-derived AGE adducts are shown in **Figure 2**.

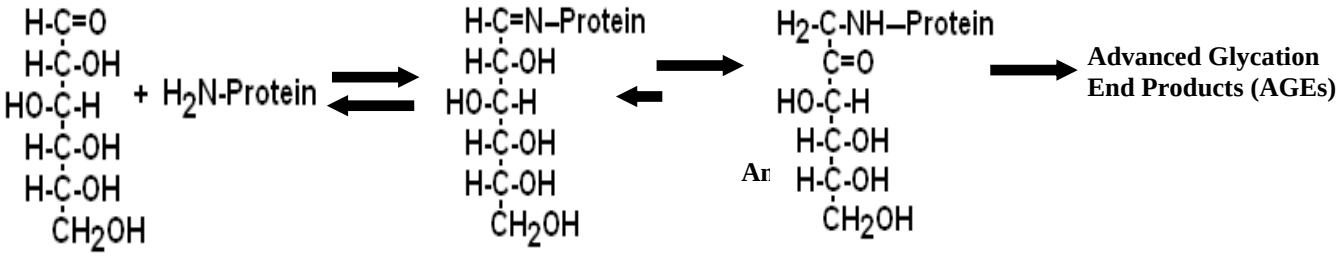

Figure 1. Non-enzymatic glycation of protein (Maillard reaction). Reducing sugars react with proteins leading to formation of advanced glycation end products (AGEs) through several steps, including Schiff's base formation, Amadori rearrangement, etc.

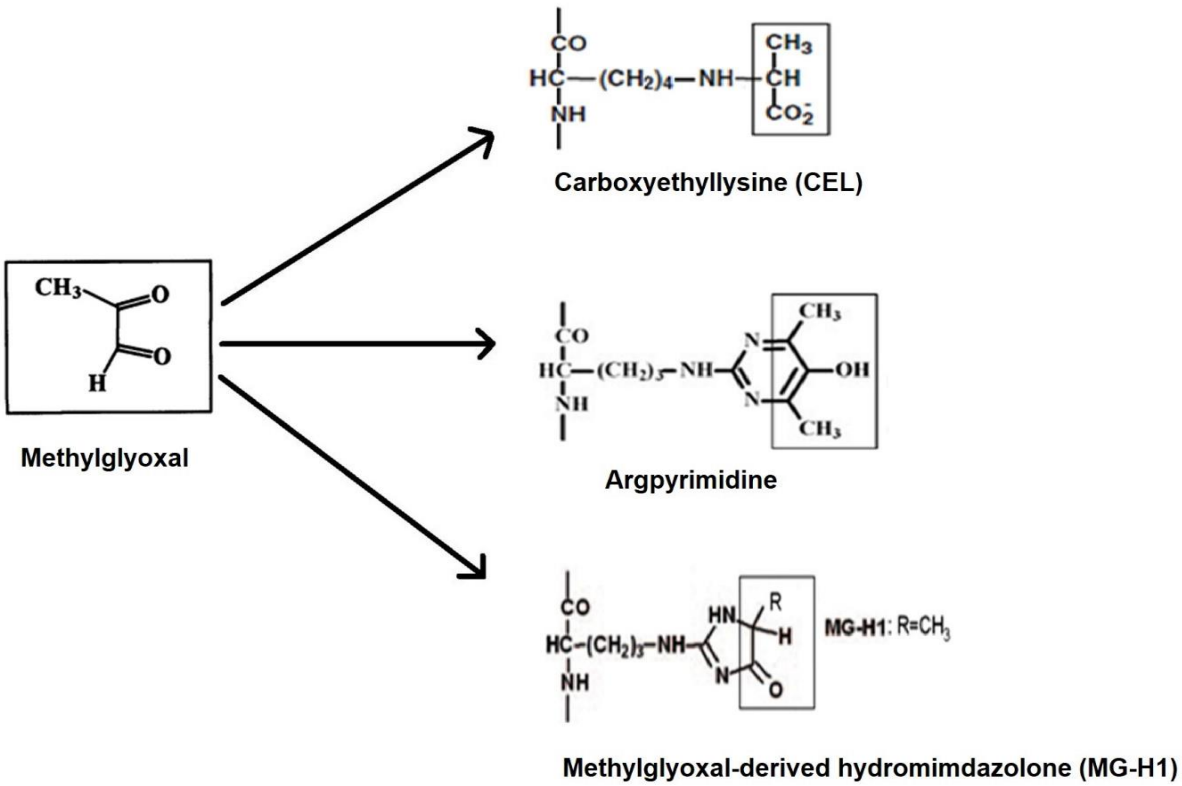

Figure 2. Chemical structures of methylglyoxal-derived AGE adduct. AGE adducts formed at lysine (N-carboxyethyllysine) or arginine residues (argpyrimidine, MG-derived hydroimidazolone) on protein are shown.

Glyoxal is a major product of glucose degradation under oxidative conditions **(Thornalley, Langborg, & Minhas, 1999)**. It is formed directly during the oxidative degradation of polyunsaturated fatty acids **(Fu et al., 1996)** and myeloperoxidase-mediated degradation of serine at sites of inflammation **(Anderson et al., 1997)**. It has been reported to interact with several proteins, namely, α-crystallin **(Kumar et al., 2004)**, bovine serum albumin **(Mikulíková, Miksík, & Deyl, 2005)**, α-synuclein **(Lee et al., 2009)**, hemoglobin **(Iram et al., 2013)**, etc. Glyoxal predominantly modifies lysine and arginine residues of proteins to form several products, such as carboxymethyllysine (CML),

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carboxymethylarginine (CMA), glyoxal-derived dihydroxyimidazolidines (GDHs; G-DH1 and G-DH2) and glyoxal-derived hydroimidazolones (G-H1, G-H2 and G-H3). Some important glyoxal-derived AGE adducts are shown in **Figure 3**.

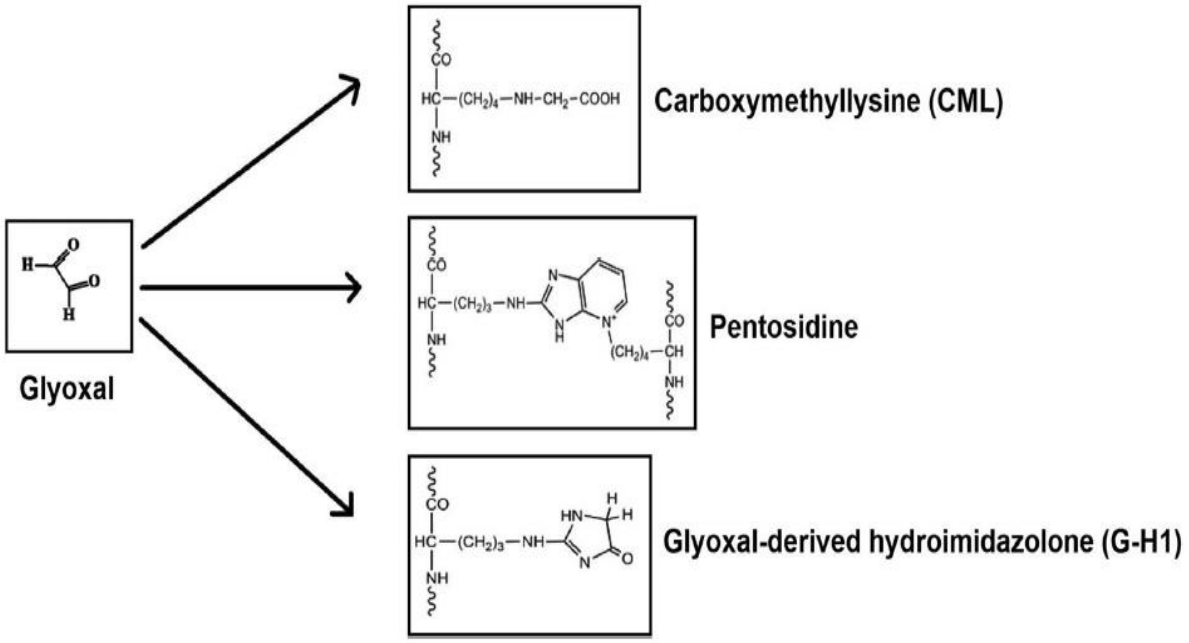

Figure 3. Chemical structures of glyoxal-derived AGE adduct. The structures of AGE adduct *N*-carboxymethyllysine, glyoxal-derived hydroimidazolone (G-H1) and pentosidine are shown.

Inhibitory role of α-oxoaldehydes on protein aggregation

In recent studies we have found that AGE modification by glyoxal and methylglyoxal increased the stability of lysozyme and provided considerable resistance to protein aggregation **(Banerjee, 2020a; Banerjee, 2020b)**. In an earlier study, methylglyoxal-induced modification of hemoglobin increased its thermal stability and provided resistance to stress-induced aggregation **(Banerjee & Chakraborti, 2017)**. Methylglyoxal modification of arginine residues decreased stress-induced aggregation of several proteins, namely, insulin, α-lactalbumin, alcohol dehydrogenase and γ–crystalline **(Biswas, Wang, Miyagi, & Nagaraj, 2008)**. Modification with glyoxal was associated with an increase in α-helical content of the protein. It is speculated that an increase in helical content due to modification could be associated with an enhancement of stability and resistance to aggregation. However, several studies have shown an increase in protein stability without change of secondary structure. The increase in thermodynamic stability of αA-crystallin was found to be associated with subtle changes of tertiary structure but without any change in secondary structure **(Nagaraj et al., 2012)**. Glyoxal or methylglyoxal induced decrease in surface hydrophobicity of lysozyme (due to tertiary structural changes) together with the formation of AGE adducts possibly affords increased resistance to aggregation.

Changes in tertiary structure and modification of positively charged residues to neutral adducts may be associated with alteration in the exposure of hydrophobic residues and decreased hydrophobic interaction of protein, thus reducing its susceptibility to aggregation. Methylglyoxal-mediated decrease in surface hydrophobicity was reported to be associated with enhanced resistance to stress-induced aggregation of proteins **(Biswas, Wang, Miyagi, & Nagaraj, 2008)**. In earlier studies, methylglyoxal modification was found to inhibit amyloid fibril formation in insulin **(Oliveira et al., 2011)** and restrict glycation-mediated loss in chaperone function in α-crystallin **(Puttaiah, Biswas, Staniszewska, & Nagaraj, 2007)**. In another study, fructose was found to restrain fibrillogenesis of albumin **(Pandey et al., 2013)**. In recent studies, glyoxal modification was reported to enhance the stability of hemoglobin and provide resistance to denaturant-induced unfolding **(Banerjee & Chakraborti, 2014**; **Banerjee, 2017)**.

Potential therapeutic possibility of α-dicarbonyls

The anti-amyloidogenic property of α-dicarbonyl compounds thus provides new insight on a possible therapeutic potential against protein conformational disorders and hereditary human lysozyme amyloidosis with clinical implications. The recent studies may therefore serve as baseline for the development of small molecule-based therapeutics in future

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for diseases attributed to protein aggregation. This can be a novel strategy to explore reactive α-oxoaldehydes to target protein aggregates in order to curb systemic amyloidosis. In a recent study, the beta-adrenergic agonist, isoprenaline hydrochloride, is reported to inhibit protein fibrillation as well as disaggregate preformed fibrils **(Chandel et al., 2019)**. Till date, only a few compounds have been reported to successfully disaggregate protein fibrils **(Konar et al., 2017; Fazili, Bhat, Bhat, & Naeem, 2016; Khan et al., 2019)**. It will be a particularly interesting approach to test the potency of the α-dicarbonyl compounds in their ability to disaggregate preformed protein fibrils. This is likely to add further as far as implications to the treatment of the disorder is concerned in addition to curbing of the same at the onset. A therapeutic strategy of lysozyme conformational disease using the α-oxoaldehydes is outlined in **Figure 4**.

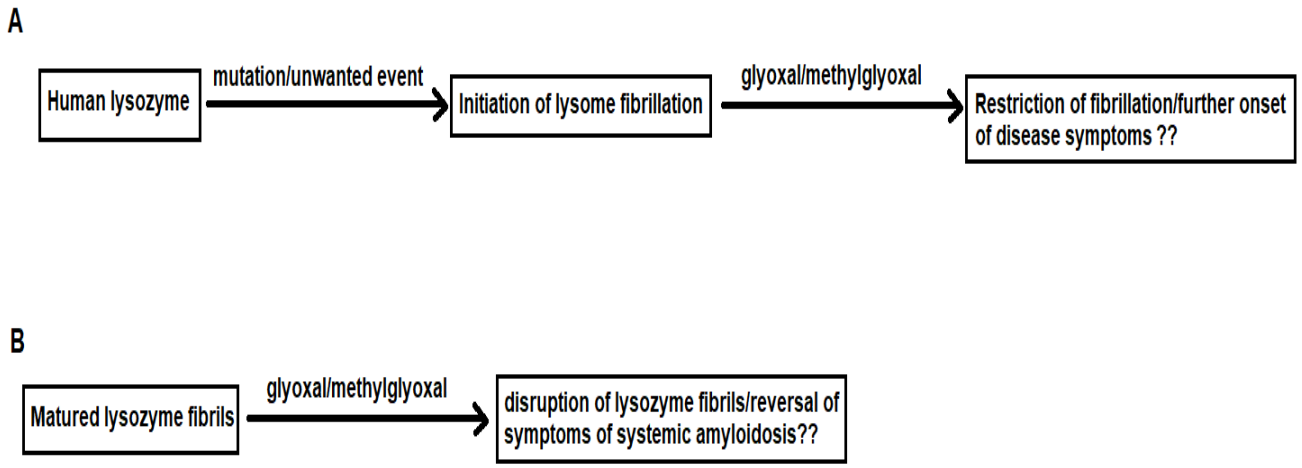

Figure 4. A possible therapeutic potential of α-oxoaldehydes in the treatment of lysozyme conformational disorder. **A.** At the initial stages of onset of fibrillation, glyoxal or methylglyoxal may restrict further fibril formation or progress of disease symptoms. **B.** The α-oxoaldehydes may also serve to disrupt already preformed fibrils and help reversing the disease symptoms acting as a curative agent for systemic lysozyme amyloidosis.

Possibility of drug repurposing

Clinical antitumor agents, doxorubicin and paclitaxel are known to exert their antiproliferative activity by increasing the cellular concentration of methylglyoxal in human host tissues. The anti-amyloidogenic property of methylglyoxal provides a mechanistic rationale for repurposing of these drugs for the treatment of systemic lysozyme amyloidosis. Thus, treatment with the drugs increasing cellular methylglyoxal may be beneficial in patients with the hereditary protein conformational disorder and may decrease the complications and improve the symptoms associated with the disorder. In conclusion, doxorubicin and paclitaxel may have therapeutic potential for the treatment of systemic amyloidosis in patients suffering from the disease. A probable mechanism of action of antitumor agents in the treatment of the hereditary disorder is outlined in **Figure 5**.

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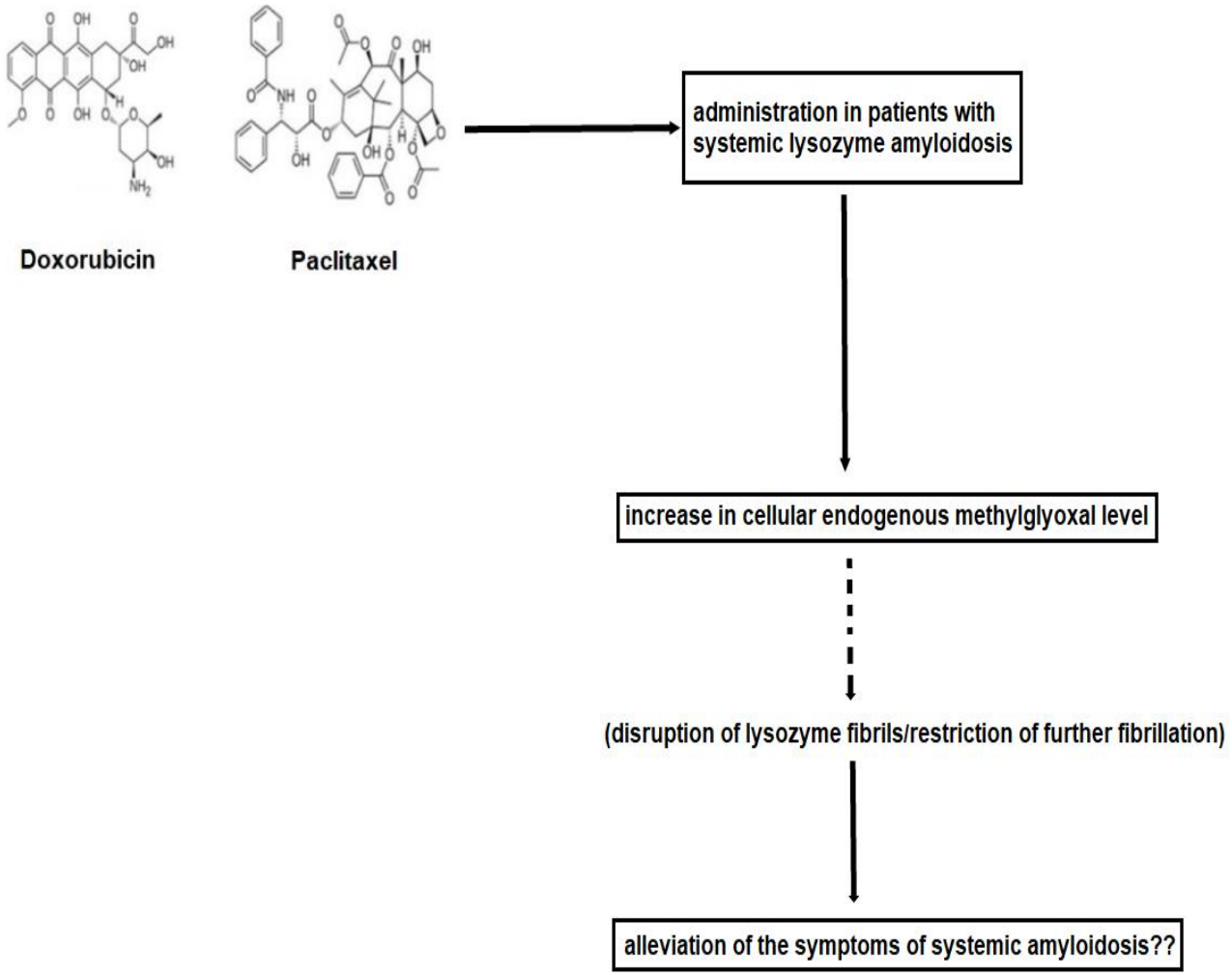

Figure 5. A possible mechanism of action of antitumor agents, doxorubicin and paclitaxel. The agents are likely to increase the level of cellular methylglyoxal which can either disrupt the preformed fibrils or restrict further fibril formation in patients in the process can act as a possible curative drug for treatment of systemic amyloidosis.

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