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A novel mechanism of methylglyoxal cytotoxicity in prostate cancer cells

Cinzia Antognelli[a], Letizia Mezzasoma[a], Katia Fettucciari[b], Vincenzo Nicola Talesa[a,*]

[a] Department of Experimental Medicine and Biochemical Sciences, University of Perugia, via del Giochetto 06122, Perugia, Italy
[b] Department of Clinical and Experimental Medicine, University of Perugia, via Enrico dal Pozzo 06122, Perugia, Italy

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ABSTRACT

Methylglyoxal is one of the most powerful glycating agents of proteins and other important cellular components and has been shown to be toxic to cultured cells. Methylglyoxal cytotoxicity appears to occur through cell-cycle arrest but, more often, through induction of apoptosis. In this study we examined whether, and through which molecular mechanism, methylglyoxal affects the growth of poorly aggressive LNCaP and invasive PC3 human prostate cancer cells, where its role has not been exhaustively investigated yet. We demonstrated that methylglyoxal is cytotoxic on LNCaP and PC3 and that such cytotoxicity occurs not via cell proliferation but apoptosis control. Moreover, we demonstrated that methylglyoxal cytotoxicity, potentiated by the silencing of its major scavenging enzyme Glyoxalase I, occurred via different apoptotic responses in LNCaP and PC3 cells that also showed a different susceptibility to this metabolite. Finally, we showed that the observed methylglyoxal apoptogenic role involved different molecular pathways, specifically mediated by methylglyoxal or methylglyoxal-derived argpyrimidine intracellular accumulation and NF-kB signaling-pathway. In particular, in LNCaP cells, methylglyoxal, through the accumulation of argpyrimidine, desensitized the key cell survival NF-kB signaling pathway, which was consistent with the modulation of NF-kB-regulated genes, triggering a mitochondrial apoptotic pathway. The results suggest that this physiological compound merits investigation as a potential chemo-preventive/-therapeutic agent, in differently aggressive prostate cancers.

1. Introduction

Methylglyoxal (MG) is an extremely reactive α-ketoaldehyde endogenously produced by various metabolic pathways, including the dephosphorylation of glycolytic intermediates, metabolites of the polyol pathway, and aminoacetone metabolism (Rabbani and Thornalley, 2012). MG is one of the most powerful glycating agents of proteins and other important cellular components (Lo et al., 1994; Vaca et al., 1994). MG glycation reactions result in the production of advanced glycation end products (AGEs). Among them, argpyrimidine (AP) represents one of the major products deriving from MG modifications of proteins arginine residues (Kim et al., 2012a,b). It has been shown that MG is toxic to cultured cells (Amicarelli et al., 2001, 1998; Kang et al., 1996; Okado et al., 1996). In this regard, MG shows significant anti-proliferative properties (Gillespie, 1975; Szent-Gyorgyi, 1968) as it can irreversibly modify nucleic acids (Amicarelli et al., 2003; Bair et al., 2010; Kang et al., 1996; Santel et al., 2008). However, it has been recently demonstrated that MG is also able to promote proliferation of vascular smooth muscle cells (Chang et al., 2011). Therefore, a cell-type specific MG proliferative effect exists, and the role of MG in cell proliferation control remains open to question. More often, MG cytotoxicity occurs through the induction of apoptosis (Chan et al., 2007; Ghosh et al., 2011a,b; Huang et al., 2011; Oba et al., 2012). There are multiple mechanisms by which MG can induce apoptosis, e.g., through the generation of reactive oxygen species (ROS) (Amicarelli et al., 2003; Chan et al., 2007; Du et al., 2001; Li et al., 2007), induction of oxidative DNA damage (Kim et al., 2011) or accumulation of a specific MG-derived AGE (Kim et al., 2010). Finally, it has been recently demonstrated that MG induces apoptosis through the inhibition of both glycolysis and mitochondrial respiration and is specific against cancerous cells (Ghosh et al., 2011a). In this regard, further experimental evidences indicated mitochondrial complex I of exclusively malignant cells as the target of MG, strongly suggesting that such a mitochondrial

Abbreviations: MG, methylglyoxal; AGEs, advanced glycation end products; AP, argpyrimidine; ROS, reactive oxygen species; p38 MAPK, mitogen-activated protein kinases; JNK, c-Jun N-terminal kinases; GLOI, Glyoxalase I; ATCC, American Type Culture Collection; RIPA buffer, radioimmunoprecipitation assay buffer; HRP, horse radish peroxidase; mAb, monoclonal antibody; ECL, enhanced chemiluminescence; TBST, tris buffer saline TWEEN-20; siGLOI, siRNA oligonucleotides targeting GLOI; siCONTROL, non-targeting siRNA oligos; qRT-PCR, Real Time TaqMan PCR analysis; TRITC, tetramethylrhodamine B isothiocyanate; FITC, fluorescein isothiocyanate; PBS, phosphate buffered saline; DAPI, 4′,6-diamidino-2-phenylindole.

* Corresponding author. Tel.: +39 075 5857483; fax: +39 075 5857483.
E-mail addresses: cinzia.antognelli@unipg.it (C. Antognelli), lmezzaso@unipg.it (L. Mezzasoma), katiafet@unipg.it (K. Fettucciari), talesa@unipg.it (V.N. Talesa).

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complex might be critically altered in these cells (Ghosh et al., 2011b). The antiproliferative and apoptogenic activity of MG has been investigated for potential pharmacological application in cancer chemotherapy (Milanesa et al., 2000), even though cells are not equally sensitive to its toxicity (Amicarelli et al., 2003; Du et al., 2000; Ghosh et al., 2011b; Talukdar et al., 2009). In addition to MG, AGEs themselves can trigger apoptosis, through increasing oxidative stress or inducing the expression of pro-apoptotic cytokines (Chuang et al., 2011; Denis et al., 2002; Kasper and Funk, 2001; Kim et al., 2012a,b; Lin et al., 2012). Although the action of MG in influencing cellular components has been studied, and amino acid residues affected by MG have been identified, detailed molecular events caused by MG, which activates the intracellular signal transduction pathway and leads the cells to apoptosis, have not yet been completely clarified (Thornalley and Rabbani, 2011). A study reported that MG-induced alterations in growth factor receptor signaling might be implicated in the development of MG cytotoxicity (Cantero et al., 2007). Another study described that MG affects cell viability via desensitization of gp130/STAT3 signaling, which is the key signaling pathway for cell survival in neuroglial cells (Lee et al., 2009). Furthermore, p38 mitogen-activated protein kinases (MAPK) activation was suggested to be a key signaling intermediate of MG-induced apoptosis in kidney cells (Liu et al., 2003) and Schwann cells (Fukunaga et al., 2005), while (Chan et al., 2007) the c-Jun N-terminal kinases (JNK) pathway appears to be important for MG-induced apoptosis in human osteoblasts. Therefore, the apoptogenic role of MG occurs in cell-type signaling pathways. In prostate cancer, the role of MG has been scarcely investigated. Two studies on the effect of MG only on PC3 cells showed that it is capable of inducing apoptosis due primarily to a blocking of the cell cycle progression and glycolytic pathway (Milanesa et al., 2000) or to a reduction in specific enzymatic activities (Davidson et al., 2002). However, to our knowledge, signaling pathways involved in MG cytotoxicity have never been investigated in prostate cancer cell models. Glyoxalase system, consisting of Glyoxalase I (GLOI, EC 4.4.1.5) and II (GLOII, EC 3.1.2.6) enzymes, represents the major cellular defence against MG- and AGEs-induced cytotoxicity (Nakadate et al., 2009; Rabbani and Thornalley, 2012). Increased expression of GLOI occurs in some tumors, such as breast and ovarian cancers (Rulli et al., 2001; Smith-Beckerman et al., 2005), prostate cancer (Davidson et al., 1999) and melanoma (Bair et al., 2010). In addition, it has been shown that GLOI is involved in resistance of human leukemia cells to antitumor agent-induced apoptosis (Sakamoto et al., 2000) and, more recently, in the survival of aggressive and invasive prostate cancer cells (Antognelli et al., 2012).

In the present work we studied whether, and through which mechanism, MG, alone or in combination with GLOI silencing, affects the growth of differently aggressive and invasive LNCaP and PC3 human prostate cancer cell lines, where its cytotoxic role has never or scarcely been investigated (Milanesa et al., 2000; Davidson et al., 2002), respectively.

2. Materials and methods

2.1. Reagents

All reagents were purchased from Sigma–Aldrich (Milan, Italy) unless stated otherwise.

2.2. Human prostate cancer cell lines and MG treatment

Human prostate adenocarcinoma LNCaP and PC-3 cells were obtained from ATCC (American Type Culture Collection) (Milan, Italy) and routinely maintained at 37 °C in 5% CO_2 in RPMI 1640 supplemented with 10% heat inactivated (1 h at 56 °C) FBS, 1× L-glutamine, 1 mM sodium pyruvate, 1× non-essential amino acids, 100 units/ml of penicillin and 0.1 mg/ml of streptomycin (Invitrogen, Milan, Italy). The cells were incubated with MG (purified by distillation before use) in appropriate cell culture conditions for 24, 48 and 72 h, in preliminary experiments, and for 72 h in the subsequent experiments.

2.3. Cell proliferation

Cell proliferation was determined by [3H]thymidine incorporation assay (Tso et al., 2000).

2.4. Cell cycle analysis and apoptosis evaluation by flow cytometry

Cell cycle distribution and apoptosis were evaluated as previously described (Antognelli et al., 2012).

2.5. TUNEL assay (ApoAlert® DNA fragmentation assay)

The nuclear DNA fragmentation was evaluated by a commercial kit (ApoAlert® DNA Fragmentation Assay, Clontech Laboratories, Inc.) in accordance with the manufacturer's instructions. The ApoAlert® DNA fragmentation assay kit detects apoptosis-induced nuclear DNA fragmentation via a fluorescence assay. The assay is based on terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end-labeling (TUNEL). TdT catalyzes incorporation of fluorescein-dUTP at the free 3′-hydroxyl ends of fragmented DNA. Fragments that incorporate the fluorescent probe can be quantified with a flow cytometer using $\lambda = 520$ nm (FITC channel). Each sample is also treated with propidium iodide (PI) fluorescent at $\lambda > 620$ nm, which gets incorporated in apoptotic and nonapoptotic cells. The percentage of green fluorescent cells and red fluorescent cells is considered as an apoptotic hallmark (Russo et al., 2010).

2.6. Whole-cell protein extraction and Western blot

For extraction of total proteins, cells were lysed in pre-cooled radioimmunoprecipitation assay (RIPA) lysis buffer (Fettucciari et al., 2006). For subcellular fractionation, cells were resuspended in Mitobuffer (Fettucciari et al., 2006). For Western blot, samples of equal protein concentration (40 μg) were treated with Laemmli buffer (Invitrogen, Milan, Italy), boiled for 5 min, resolved on 10, 12 or 15% SDS-PAGE and then blotted onto a nitrocellulose membrane, using iBlot Dry Blotting System (Invitrogen Life Technologies, Milan, Italy). Non-specific binding sites were blocked in Roti-Block (Roth, Germany) for 1 h at room temperature, incubated overnight at 4 °C with an appropriate dilution of the primary specific Abs (mouse anti GLOI mAb, BioMac, GmbH, Leipzig, Germany; mouse anti-AP mAb, Antibodies-online, GmbH, Aachen, Germany; mouse anti-β-actin mAb, rabbit anti-Bcl-XL polyclonal Ab, rabbit anti-Bax (N20)polyclonal Ab, Santa Cruz Biotechnology, Heidelberg, Germany; rabbit anti caspase-3 polyclonal Ab, rabbit anti phospho-I-kappa-B-alpha (Ser32) (14D4) and anti I-kappa-B-alpha (44D4) mAbs, Cell Signaling Technology, Milan, Italy; mouse anti-Bcl-2 mAb, DAKO, Milan, Italy; mouse anti-Cytochrome c (Cyt c) mAb, BD Pharmingen, Milan, Italy; mouse anti-Cyt c oxidase subunit IV (Cox IV) mAb, Molecular Probes, Monza, Italy). After washing with TBST, antigen–antibody complexes were detected by incubation of the membranes for 1 h at room temperature with the appropriated HRP-conjugated secondary Ab and revealed using ECL system (Amersham Pharmacia, Milan, Italy). Densitometry analyses were performed in ImageJ software. As internal loading controls and for protein expression normalizing purpose, all