The temporal and spatial dynamics of glyoxalase I following excitoxicity and brain ischaemia

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Abstract

MG (methylglyoxal) is an inevitable metabolite derived from glycolysis leading to protein modification, mitochondrial dysfunction and cell death. The ubiquitous glyoxalase system detoxifies MG under GSH consumption by mean of Glo1 (glyoxalase I) as the rate-limiting enzyme. Neurons are highly vulnerable to MG, whereas astrocytes seem less susceptible due to their highly expressed glyoxalases. In neurodegenerative diseases, MG and Glo1 were found to be pivotal players in chronic CNS (central nervous system) diseases. Comparable results obtained upon MG treatment and NMDA (*N*-methyl-D-aspartate) receptor activation provided evidence of a possible link. Additional evidence was presented by alterations in Glo1 expression upon stimulation of excitotoxicity as an event in the aftermath of brain ischaemia. Glo1 expression was remarkably changed following ischaemia, and beneficial effects were found after exogenous application of Tat (transactivator of transcription)–Glo1. In summary, there are strong indications that Glo1 seems to be a suitable target to modulate the consequences of acute neuronal injury.

The glyoxalase system and the relationship to diseases

The glyoxalase system comprising Glo1 (glyoxalase I; EC 4.4.1.5) and Glo2 (glyoxalase II; EC 3.1.2.6) is the main detoxifying system of MG (methylglyoxal) within all mammalian cells [1]. The dicarbonyl MG is primarily generated by the spontaneous degradation of triosephosphates as a by-product of glycolysis.

MG reacts with reduced glutathione (GSH) to a hemithioacetal which is subsequently converted into *S*-D-lactoylgluthatione by the rate-limiting enzyme Glo1 and metabolized further to D-lactate by Glo2, restoring the used GSH. Glo1 is a zinc metalloenzyme with a molecular mass of 42 kDa consisting of two similar monomers with a molecular mass of 21 kDa [1].

In many studies, the cytotoxic effects of enhanced MG levels such as DNA breakage, cytokine production/release, ROS (reactive oxygen species) production, mitochondrial dysfunction and related caspase activation, as well as protein glycation and associated functional impairment, have been found [1–6].

Regarding these effects, changes in MG and Glo1 indicate a possible role in the development of diseases. Actually, Glo1 alterations are documented for diabetes, aging, tumorigenesis, multidrug-resistance and nephropathy [7–10].

Key words: excitotoxicity, glyoxalase I, methylglyoxal, neuronal injury, stroke ischaemia.
Abbreviations: BBB, blood–brain barrier; CNS, central nervous system; $GABA_AR$, γ-aminobutyric acid A receptor; Glo, glyoxalase; MG, methylglyoxal; NMDA, *N*-methyl-D-aspartate; OHSC, organotypic hippocampal slice culture; Tat, transactivator of transcription.
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The fourth highest Glo1 level was detected in the brain, underlining a pivotal role of this enzyme within the CNS (central nervous system) [11].

Changes in Glo1 activity and expression or elevated MG levels are associated with anxiety, schizophrenia, autism, restless-legs syndrome, epilepsy, Alzheimer's disease and Parkinson's disease [12–14]. Furthermore, the relationship between Glo1 changes and behavioural disorders is a matter of controversy, since results from animal experiments seem not to match with human genetic studies [12].

All of the aforementioned diseases are chronic and only a few reports of Glo1 and/or MG in acute brain pathologies are available [15,16]. The role of Glo1 and MG in these events is still not completely elucidated. But some reports indicate that the glyoxalase system and its substrates might be suitable therapeutic targets in the aftermath of brain ischaemia and related events such as excitotoxicity.

Neurotoxicity, excitotoxicity and their relationship to MG and Glo1

MG treatment leads to neurotoxicity in a time- and concentration-dependent manner by the aforementioned mechanisms as well as the inhibition of Glo1 [5,6,17–19].

It has to be noted that the effects of MG differ substantially during development and between cell lines [5,19]. Furthermore, in cell lines, the state of cell differentiation might be crucial for the different vulnerabilities to MG. Additionally, the use of different concentrations of MG (100–1000 μM) may also be the cause of the reverse effects observed on the glyoxalase system [5,6,19].

Primary neurons behave similarly to the SH-SY5Y cell line when treated with MG [20]. Moreover, in primary neurons, MG increased the expression of the survival factors NGF (nerve growth factor) and BDNF (brain-derived neurotrophic factor) [3,4,21]. But these effects were not sufficient to protect neurons from MG-mediated cell death. In parallel, the accelerated neurodegeneration seems to be associated with MG-induced expression of inflammatory cytokines such as IL-1β (interleukin 1β) and TNFα (tumour necrosis factor α). But, so far, from a methodological point of view, it should be considered that Glo1 differs during brain development and the aforementioned studies used cultures from fetal rat brains [22]. Therefore MG-triggered effects may occur more pronounced compared with cell cultures of postnatal animals or *in vivo*.

After MG treatment, astrocytes displayed a higher viability and ability to protect neurons from MG toxicity [23]. Higher protein levels and activities of Glo1 and Glo2 therefore indicate a higher capacity to metabolize MG.

Enhanced glutamate levels were measured in SH-SY5Y cells after MG treatment, providing a possible link between MG toxicity and excitoxicity and related cell death [17]. The pathological activation of the NMDA (*N*-methyl-D-aspartate) receptor, an ionotropic glutamate receptor, is marked by an excessive influx of cations, mainly Ca^{2+} ions, contributing to free radical production in mitochondria, caspase activation and cytokine release [24–28]. The inhibition of the NMDA receptor using specific antagonists attenuated MG-induced glutamate enhancement in SH-SY5Y cells and ameliorated cell survival [17]. Recently, MG has been identified as a partial agonist of $GABA_AR$s (γ-aminobutyric acid A receptors) without directly affecting glutamate receptors [29]. The function of MG as a $GABA_AR$ agonist would change the role of MG from a destructive to a potential protective player in excitotoxicty by counteracting the excessive depolarization of glutamatergic neurons [30]. In this context, enhanced Glo1 levels were found to induce a higher excitability of neurons. However, in an another study, neither silencing nor overexpression of Glo1 affected the susceptibility to 10 μM glutamate [23]. The glutamate concentrations used might be too low to induce excitotoxic events and may therefore explain the lack of effect [31].

Since the temporal and spatial dynamics of Glo1 in excitotoxicity were not investigated previously, we analysed in our very recent study changes in Glo1 using the *in vitro* model of OHSCs (organotypic hippocampal slice cultures) lesioned with NMDA [15]. This model is well accepted for examining pharmacological and biochemical effects as well as for morphological changes in excitotoxicity [32,33].

We detected the Glo1 monomer at 23 kDa and the Glo1 dimer at 46 kDa in OHSCs [15]. In untreated OHSCs, no significant changes in Glo1 could be determined over the period of investigation. After an excitotoxic insult, a significant increase in the level of Glo1 dimer at 12–24 h, as well as a significant decrease in the level of Glo1 monomer at 48 h, were found when compared with unlesioned time-matched controls. In addition, significant differences in the levels of the Glo1 dimer and monomer were determined 24–72 h after the lesion. Hence a possible redistribution between monomer and dimer was assumed, and a ratio calculated. Ratios of the control group revealed no significant changes over the time. In contrast, in the excitotoxically lesioned group, we found a significant increase between 12 and 24 h.

The nature and function of the observed Glo1 dimer remains unexplained. The appearance might be due to a post-translational modification caused by an elevated transglutaminase activity after NMDA receptor activation, leading to a cross-linking which rendered modified proteins insoluble even after treatment by reducing agents such as 2-mercaptoethanol [34,35].

The spatial distribution of Glo1 was analysed in the dentate gyrus. In control slices, astrocytic processes mainly displayed Glo1 immunoreactivity. However, in the NMDA-treated group, the pattern of Glo1 immunoreactivity changed over time.

Interestingly, within the first 24 h after the excitotoxic insult, we observed a translocation of Glo1 immunoreactivity from neuronal cytosol to the cell membrane, whereas these changes were not present in controls.

MG and Glo1 in brain ischaemia

Stroke is a frequent cause of death whereby the ischaemic type (approximately 60%) seems to be the most common subtype [36].

Vessel occlusion leads to a decreased blood flow impairing neuronal metabolic functions causing energy failure and cell death [37]. Only thrombolysis within 3 h is believed to ameliorate the neurological outcome after stroke, whereas available neuroprotective drugs had no clinical effect [37]. Nevertheless, infarct size and neurological impairment increase after initial insult. This might be a consequence of secondary neuronal injury that is induced by necrotic and apoptotic cell death and leads to neuroinflammation [38,39]. In contrast with primary injury which takes minutes, the secondary processes may be extended to weeks after CNS injury [40].

In our recent study, we performed permanent middle cerebral artery occlusion and observed temporal and spatial changes in Glo1 immunoreactivity [15]. Until 75 min after ischaemia, Glo1 immunoreactivity was found in the endothelium of blood vessels on both ipsilateral and contralateral sites with a stronger intensity ipsilaterally. On the basis of this observation, we postulated a possible defence mechanism against the breakdown of BBB (blood–brain barrier) by Glo1 up-regulation. As the rupture of BBB is the critical limiting factor for the induction of thrombolysis, the protection of BBB is of vital interest [41]. The exposure of immortalized brain microvascular cells to MG in a two-chamber model has been reported to result in barrier dysfunction observed by an increased flux of proteins to the chamber beyond the immortalized brain microvascular cells [42]. The use of edaravone, a radical scavenger used in ischaemic therapy in Asia, prevented these effects [43].

In the aftermath of ischaemia from 1 to 10 days, neurons at the ipsilateral site especially showed Glo1-immunoreactivity, whereas an endothelial pattern was found at the contralateral site [15]. At 60 days after ischaemia, the neuronal labelling pattern disappeared and astrocytes of the astrocytic scar displayed a strong Glo1-immunoreactivity.

In other systems, especially the kidney, decreased Glo1 activity and increased MG adducts were found 24 h after transient renal ischaemia [44]. Glo1 overexpression decreased cell death and MG adducts after ischaemia.

In summary, we determined Glo1 as a major player in the aftermath of brain ischaemia; however, its nature has to be determined in further studies. In a very recent study, Glo proteins were coupled to the Tat (transactivator of transcription) peptide as a vehicle to provide Glo1 and Glo2 exogenously to cells of the CNS. These Tat–Glo proteins were intraperitoneally injected 30 min before performing transient forebrain ischaemia in gerbils [16]. Increased intracerebral Glo levels were detected, indicating the ability of the agent to pass the BBB. At 7 and 14 days after ischaemia, cell viability was measured and Tat–Glo proteins were found to be neuroprotective. Interestingly, the simultaneous injection of Tat–Glo1 and Tat–Glo2 displayed the highest protection.

Astrocytes play a dual role and are able to decrease or increase neuronal injury [39,45]. The astrocytic scar restricts the expansion of injury, but, at the same time, it can impair neuronal remodelling by reducing neurite outgrowth [46]. There are no reports on the relevant role of Glo1 or MG in astrogliosis. Indirectly, the use of ethyl pyruvate, a known Glo1 inhibitor of Glo1, led to a decelerated astrocytic scar formation ([47,48], and P. Pieroh, G. Birkenmeier and F. Dehghani, unpublished work).

Conclusion and outlook

In addition to the relevant implications of Glo1 in chronic neurodegenerative disorders, recent studies have also revealed a pivotal role of Glo1 in acute neuronal injuries. These changes displayed spatial and temporal dynamics which provide Glo1 as suitable targets to decrease neuronal loss in the aftermath of excitotoxicity and brain ischaemia. Moreover, alterations in Glo1 levels make the protein interesting as a potential marker to follow the time course and the extent of neuronal injury. However, some questions remain open. Particularly, the protective or destructive nature of Glo1 is not clearly elucidated. In addition, further investigations are needed to clarify the up- and down-stream mechanisms which affect the glyoxalase system within cells of the CNS. Furthermore, it has to be shown whether the up-regulation of Glo1 is an ischaemia-specific intrinsic phenomenon or whether it may also occur in other acute CNS lesions such as traumatic brain injury. Also, studies focusing on the role of MG in distinct CNS cells are desirable. Besides the toxicity of MG to neurons, it is necessary to characterize the MG effects in astrocytes or microglia comprehensively, especially with attention to their contribution to neuroinflammation and immunomodulation.

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