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Research Report

Oxidative damage is present in the fatal brain edema of diabetic ketoacidosis

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ABSTRACT

Oxidative stress is implicated as a pathogenic factor in a spectrum of chronic diseases, notably, neurodegenerative disease. Noteworthy in this regard is that type 1 diabetes mellitus (T1DM) results in oxidative stress, leading to systemic complications of T1DM. We hypothesized that oxidative stress associated with diabetic ketoacidosis (DKA) of T1DM might have measurable brain sequelae. Consistent with this hypothesis are neurohistology and neuroradiologic studies of T1DM that suggest oxidative insults are involved in the chronic complications of diabetic encephalopathy. To further address the role of oxidative stress in an acute setting, specifically in fatal brain edema (BE) associated with DKA, we studied neuronal localization and levels of oxidative stress markers reported to be increased in other neurodegenerative conditions. We demonstrated increased levels of 8-hydroxyguanosine (8OHG), 4-hydroxynonenal (HNE), and heme oxygenase-1 (HO-1) in the pyramidal neurons of the hippocampus of DKA BE in comparison to controls. However, in the cerebellum, only 8OHG was increased in the Purkinje cells and other cells of the molecular layer. These results indicate a role for oxidative stress in the pathogenesis of T1DM encephalopathy.

1. Introduction

Limitations in the physiological control of type 1 diabetes mellitus (T1DM) result in varying degrees of hypoglycemia, hyperglycemia, and ketosis, each of which is a metabolic mediator of oxidative stress (Jain et al., 1999; Singh et al., 2004; Ceriello, 2006). As such it is not surprising that the deleterious effects of oxidative stress have a cumulative effect that is related to the duration of T1DM (Martin-Gallan et al., 2007). Such oxidative changes are recognized in the pathogenesis of the chronic vascular complications and peripheral neuropathy of diabetes (Baynes, 1991; Ceriello, 2006). Likewise, evidence for oxidative stress and neuroinflammation is also described in the pathogenesis of diabetic encephalopathy (Mastrocola et al., 2005; Hoffman et al., 2008; Sima et al., 2009a, b). Additionally, the metabolic crisis of diabetic ketoacidosis (DKA) and its treatment accentuate systemic oxidative stress and a systemic inflammatory response (Jain et al., 1999; Vantyghem et al., 2000; Lee et al., 2002; Dalton et al., 2003; Hoffman et al., 2003a,b; Jerath et al., 2005; Turk et al., 2006).

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The systemic insults associated with DKA and its treatment occur in parallel with the progression of subclinical brain edema (BE) and development of clinical BE (Hoffman et al., 1988; Durr et al., 1992). Clinical BE, a potentially fatal complication of DKA (Edge et al., 2001), is associated with disruption of tight junction proteins of the blood brain barrier (Hoffman et al., 2009).

Several characteristics make the brain susceptible to oxidative/nitrosative stress, including a high metabolic rate and oxygen requirement (Kennedy and Sokoloff, 1957); a high concentration of polyunsaturated fatty acids that increase susceptibility to lipid peroxidation (Adibhatla and Hatcher, 2008); high levels of transition metals, capable of catalyzing reactive oxygen and nitrogen species (Halliwell, 1992; Smith et al., 1994a age-dependent differences in antioxidant reserves (Bayir et al., 2006). In TIDM, insulin and insulin growth factor-1 (IGF-1) deficits in the brain result in disordered insulin signaling, deficiencies in the protein kinases Akt and GSK3, compromised brain energy metabolism, and oxidative stress (Steen et al., 2005; Li et al., 2007; Jolivalt et al., 2008; Sima et al., 2009a,b).

The role of oxidative stress in the pathogenesis of DKA/BE is incompletely understood. To address the cellular localization and levels of 8-hydroxyguanosine (8OHG), a marker of oxidative damage to RNA, the lipid peroxidation adduct 4-hydroxynonenal (HNE), the oxidatively induced enzyme heme oxygenase-1 (HO-1) and ceruloplasmin, the acute phase protein that is a transporter of iron, and an antioxidant were measured. In addition, phosphorylated p38, redox-active iron, mitochondrial DNA (mtDNA) deletions, neuronal morphology, and neuronal density were also studied.

2. Results

2.1. *Immunohistochemistry*

Within the hippocampus, products of oxidative damage accumulated at higher levels in the pyramidal neurons in both cases of DKA/BE compared to controls. 8OHG was localized to the neuronal cytoplasm and nucleoli at much higher levels in the two DKA/BE cases (Figs. 1A and B), with the control cases showing only background levels (Fig. 1C). Consistent with this, quantification showed that the 8OHG was significantly higher in the DKA/BE cases than controls ($p<0.001$, Fig. 1D). In the cerebellum, differences of 8OHG were found between the DKA/BE cases and the control cases. Both DKA/BE cases showed high levels of 8OHG in the Purkinje cells and other cells within the molecular layer (Fig. 1E and F). Comparatively, control cases had much lower amounts of 8OHG in cells (Fig. 1G). Quantification revealed that the levels of 8OHG in both the Purkinje cells as well as the other cells within the molecular layer were significantly higher ($p<0.001$, Fig. 1H) in DKA/BE compared to the controls.

HNE adducts were also increased in the pyramidal neurons of the hippocampus in the DKA/BE cases compared to control cases (Fig. 2A and B, respectively). As expected, quantification of the neuronal HNE levels revealed significantly higher levels in the DKA/BE cases compared to controls ($p<0.05$, Fig. 2C). The cerebellum of the DKA/BE cases as well as controls demonstrated only background levels of HNE adducts (data not shown). In all cases, the large vessel walls, known sites of normally occurring HNE accumulation, were positively immunostained (data not shown).

HO-1 levels were also increased in the same population of neurons in the hippocampus of the DKA/BE cases compared to controls (Fig. 3A and B, respectively). The HO-1 cytoplasmic localization in the pyramidal neurons was significant in the DKA/BE cases ($p<0.001$, Fig. 3C). In the cerebellum, HO-1 showed no differences between DKA/BE cases (Fig. 3D) and controls (Fig. 3E), and upon quantification, the Purkinje cells displayed similar levels of HO-1 (Fig. 3F).

In the pons, no differences were noted with the oxidative stress markers between DKA/BE and controls.

In the occipital cortex, ceruloplasmin (Cp) was localized to pyramidal neurons (arrows) in the DKA/BE cases (Fig. 4B), while in controls, Cp was not increased in the neuronal cell bodies (Fig. 4A) Pyramidal neurons in the occipital cortex from the cases of DKA/BE showed the highest level of Cp (Fig. 4B, arrowheads), a finding that was not seen in the controls.

Other markers of oxidative damage were analyzed in some areas of the DKA/BE cases. Neither phosphorylated p38, redox-active iron, nor mtDNA deletion *in situ* hybridization (data not shown) showed any specific accumulation in the DKA/BE cases with our methodologies.

2.2. *Quantitative analysis*

Morphological analysis of cresyl violet stained hippocampus sections revealed that the mean neuronal densities were lower in the DKA/BE cases compared to controls confirming previous reports (Hoffman et al., 2008). (187 ± 53 versus 304 ± 59 neurons/mm^2, $p<0.01$). However, no significant differences in the size of the pyramidal neurons in the hippocampus were found between the cases of DKA/BE and controls.

3. Discussion

There is convincing evidence that the bases of both messenger RNA and ribosomal RNA (Nunomura et al., 2009) are susceptible to sublethal oxidation insults (Nunomura et al., 1999, 2007; Castellani et al., 2008). 8OHG, an oxidized nucleoside derived from RNA, identifies vulnerable/damaged neurons at an early stage of several neurodegenerative diseases (Sayre et al., 2008). This study of well-recognized markers of oxidative stress in the brains of young patients with poorly controlled T1DM that included recurrent episodes of DKA and the fatal BE of DKA supports and extends the vulnerability of the brain to oxidative stress and its potential importance in the neuronal dysfunction associated with severe abnormalities in glucose and insulin metabolism. The findings that oxidative damage was mediated by insulin deficiency/resistance and neuroinflammation, as illustrated here, raise the possibility that these mechanisms underlie the pathogenesis of both acute and chronic cerebral complications, including diabetic encephalopathy (Sima and Li, 2005; Li et al., 2007; Hoffman et al., 2008, 2010; Sima et al., 2009a,b).

HNE, a biologically active carbonyl derived from polyunsaturated fatty acid peroxidation, is a signaling molecule at subtoxic levels (Dwivedi et al., 2007) and neurotoxic at higher