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BRAIN RESEARCH

Research Report

Cold-inducible RNA binding protein inhibits H_2O_2-induced apoptosis in rat cortical neurons

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ABSTRACT

Objective: The expression cold-inducible RNA-binding protein (CIRP) is significantly enhanced in neurons under hypothermia, but its roles remain unclear. This study aims to investigate whether the cerebral protection under hypothermia is mediated by the CIRP-mediated inhibition of neuronal apoptosis. Methods: Primary rat cortical neurons were isolated, cultured, and transduced with lentiviral CIRP-RNAi. Apoptosis of the transduced neurons was induced with 100 μmol/L H_2O_2, the treated cells were divided into two groups, and cultured in 37 °C or 32 °C incubator respectively. Cell viability was detected by MTT colorimetric assay. Neuronal apoptosis was detected by flow cytometry after labeling the cells with Hoechst 33342 and Annexin V-FITC/PI. The protein expressions of CIRP, activated caspase-3, and thioredoxin (TRX) were detected by Western blot. Results: Under 32 °C, CIRP protein is significantly induced in cortical neurons; the expression of activated caspase-3 decreases, while the TRX expression increases. The rate of neuronal apoptosis is 4.5±0.8%. Under 37 °C, CIRP expression is evidently reduced in cortical neurons; the expression of activated caspase-3 is significantly enhanced with reduced level of TRX expression. The rate of neuronal apoptosis reaches 53.5±1.7% ($P<0.05$, compared to that in 32 °C group). Conclusions: The induction of CIRP protein in rat cortical neurons under hypothermia inhibits H_2O_2-induced neuronal apoptosis and thereby exerts neuroprotective effect, which forms one of the cerebral protective pathways under hypothermia.

1. Introduction

Hypothermia is widely recognized as the most effective neuroprotective therapy and has been already applied clinically. However, the significant side effects of inducing complications such as hypotension, low blood volume, bradycardia, electrolyte imbalance, hyperglycemia, etc. (Clifton et al., 2001), have limited its clinical applications. The mechanisms of the protective effect of hypothermia may include the following: reducing the release of excitatory amino acids; inhibiting calcium influx, regulating the activities of calmodulin kinase II and protein kinase C and thereby ameliorating the inflammatory response after cerebral ischemia; inhibiting the formation of brain edema; reducing the rate of oxygen metabolism and the generation of free radicals; and inhibiting the neuronal apoptosis induced by mitochondrial release of cytochrome C (CytC) (Deng et al., 2004; Sakurai et al., 2006). It has been observed in recent years that the expression of certain cold shock proteins is significantly upregulated under hypothermia, though their exact functions remain unknown

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(Beer et al., 2003), which has aroused great interest among medical investigators.

It is commonly considered that the protective effects of hypothermia on corresponding tissues and organs are mediated by inhibiting the body's metabolism, reducing protein synthesis, and suppressing the body's oxygen consumption (Lleonart, 2010; Sinclair and Andrews, 2010). However, studies have shown that even under hypothermia, the expression of certain proteins, such as CIRP, has been significantly upregulated (Nishiyama et al., 1997). These results have also been confirmed in our preliminary experiments (Liu et al., 2010; Xue et al., 1999). The present study has shown that CIRP is a stress responsive protein, which has a special internal ribosome entry site (IRES)-mediated translation mechanism. Extensive investigations have demonstrated that under hypothermia, CIRP is phosphorylated by GSK3β kinase or methylated at its C-terminal region and hence transfers from the nucleus into the cytosol (De Leeuw et al., 2007; Yang et al., 2006), through the IRES translation mechanism (Al-Fageeh and Smales, 2009; Ohlmann et al., 2000) and in combination with different transcription factors, to regulate gene expression at post-transcriptional and translational levels. CIRP binds to mRNA's 5′/3′ UTR (untranslated region) to regulate the translation initiation rate, increase the stability of transcripts, and thereby help the cells to quickly adapt to environmental changes. For example, CIRP can specifically bind to the 3′-UTR of TRX mRNA to enhance its expression and thereby exert its cell protective effect (Park et al., 1999; Welsh et al., 2002; Yokomizo et al., 1995). Therefore, the authors hypothesize that CIRP executes its cell protective roles under hypothermia by translocation from nucleus to cytoplasm to promote the expression of certain proteins (e.g., TRX, etc.) through the IRES pathway, and thereby to lower oxidative stress, inhibit apoptosis signaling pathway, and help the cells to rapidly adapt to environmental changes. The present study aims to investigate and confirm the above hypothesis.

2. Results

2.1. *The relationships between hippocampal neuronal damage and the dose and duration of H_2O_2 treatment*

The concentration- and time-dependent effects of H_2O_2 on neuronal damages have been detected based on the survival rate of hippocampal neurons using MTT assay. Fig. 1 shows the relationship curves of the concentration and treatment time of H_2O_2 with their effects as reflected by the survival rate of neurons. As shown in Fig. 1, with the treatment prolonging and the dose increasing, neuronal damages aggravate, indicating a clear time- and dose-dependent effect of H_2O_2 treatment. Based on the curves, the combination of 100 μmol/L H_2O_2 and treatment for 2 h is chosen for subsequent experiments.

2.2. *CIRP expression in each group*

The expression of CIRP in each group has been detected by Western blot. As shown in Fig. 2, CIRP expression is low in normal control group, while it is clearly increased in hypothermia groups. The addition of CIRP-RNAi lentivirus has significantly inhibited the CIRP induction, while CIRP expression in hypothermia group infected with control lentivirus exhibits no significant difference from that in hypothermia control group.

2.3. *The effect of CIRP expression on H_2O_2-induced neuronal apoptosis*

After staining the cell nuclei with Hoechst 33342, evident apoptosis is observed in normal control group (Fig. 3A), while neuronal apoptosis is significantly less in hypothermia control group (Fig. 3B). When CIRP expression is inhibited by the infection of CIRP-RNAi lentivirus, neuronal apoptosis becomes

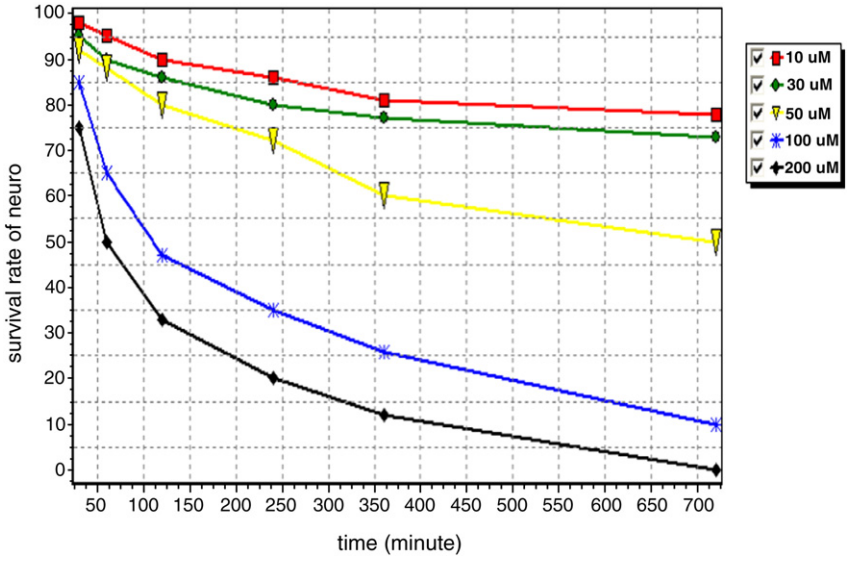

Fig. 1 – The time- and concentration-dependent effect of H_2O_2 on neuronal damage.