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BRAIN RESEARCH

Research Report

The adenoviral vector-mediated increase in apurinic/apyrimidinic endonuclease inhibits the induction of neuronal cell death after transient ischemic stroke in mice

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ABSTRACT

Despite the correlation between changes in the levels of apurinic/apyrimidinic endonuclease and ischemic neuronal damage, no studies have addressed the question of whether increased APE/Ref-1 can prevent ischemic neuronal cell death in vivo. Using an adenoviral vector, we investigated whether increased APE/Ref-1 can inhibit the loss of APE/Ref-1 and thereby prevent oxidative DNA damage after transient focal cerebral ischemia. Mice were subjected to intraluminal suture occlusion of the middle cerebral artery for 1 h, followed by reperfusion. Pre-ischemic treatment of the adenoviral vector was introduced intracerebrally. An adenoviral vector harboring the entire APE/Ref-1 gene sequence or a control virus without the APE/Ref-1 sequence was introduced 3 days before ischemia/reperfusion (I/R). The reduction of APE/Ref-1 occurred before DNA fragmentation, which was shown by temporal and spatial analysis. Increased APE/Ref-1 significantly decreased DNA damage and infarct volume after I/R. In conclusion, increased APE/Ref-1 enhanced DNA repair and inhibited the induction of ischemic oxidative DNA damage and cerebral infarction after I/R.

1. Introduction

Apurinic/apyrimidinic endonuclease/redox effector factor-1 (APE/Ref-1) is a multifunctional enzyme in the BER pathway responsible for repairing apurinic/apyrimidinic (AP) sites in DNA, which are generated by oxidative stress after ischemia/reperfusion (I/R) (Bernstein et al., 2002; Chan, 2001; Evans et al., 2000; Fishel et al., 2007). AP sites are toxic to the cell, most likely through initiating various cell-killing signaling pathways (Hanna et al., 2004; Li et al., 2006). AP sites, upon accumulation, prevent DNA synthesis or gene transcription in cells at the lesion site, directly causing cell death (Lan et al., 2003). Thus, accumulation of AP sites is an important contributing factor to ischemic neuronal cell death (Li et al., 2006). AP sites occur in the early stage after transient cerebral ischemia, and they are repaired by the enzyme APE/Ref-1 that prevents ongoing cell death (Chen et al., 1997; Lan et al., 2003; Liu et al., 1996). However, continuous or severe damage beyond the repair capabilities of the repair enzyme ultimately results in DNA fragmentation and cell death (Fujimura et al., 1999a; Kawase et al., 1999; Rich et al., 2000; Zhou and Elledge, 2000).

A number of previous in vivo studies have shown that APE/Ref-1 decreases markedly in the brain after severe focal

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ischemia and subsequent DNA fragmentation, suggesting that decreased levels of APE/Ref-1 and the failure to repair DNA may contribute to irreversible tissue injury (Chang et al., 1999; Fujimura et al., 1999a; Lan et al., 2003; Walton et al., 1997). On the contrary, sub-lethal levels of oxidative stress induce upregulation of APE/Ref-1, which suggests that this protein has a protective effect against lethal doses of oxidative stress and DNA damage (Grosch et al., 1998; Li et al., 2006; Ramana et al., 1998; Sugawara et al., 2001). However, there have been no reports in vitro or in vivo showing direct evidence for neuronal rescue by enhanced DNA repair activity after cerebral ischemia. It is also not clear that an increase of APE/Ref-1, which may increase DNA repair activity, protects from inducing neuronal cell death after ischemic insult (Evans et al., 2000). We hypothesized that upregulation of APE/Ref-1 would be able to rescue cells from oxidative DNA damage after cerebral ischemia. To test our hypothesis, we upregulated the expression of APE/Ref-1 using an adenovirus, a well-known DNA transfer vector.

In this study, we focused on DNA repair as an APE/Ref-1 repair activity and investigated whether adenoviral-vector-mediated APE/Ref-1 (Adv-APE/Ref-1) could inhibit the induction of oxidative DNA damage and prevent neuronal cell death after I/R in mice.

2. Results

2.1. *Physiological data and regional cerebral blood flow (RCBF) parameters*

Changes in RCBF were measured indirectly by laser Doppler flowmetry (Transonic Systems Inc., Ithaca NY 14850, USA); RCBF decreased after ischemia compared with preischemia and the contralateral side. There were no statistically significant differences in RCBF during ischemia between the group before occlusion and the group after reperfusion (Table 1). Physiological values were as follows in Table 1. There was no deviation from these values over the period of assessment. Intracerebral administration of Adv-APE/Ref-1 therefore does not alter the physiology of mice.

2.2. *Spatial and temporal relationship between APE/Ref-1 and AP sites after focal cerebral I/R*

APE/Ref-1 immunoreactivity was evident as a single band of 37 kDa, peaking at 30 min and decreasing from 1 to 24 h in mice brains after I/R, as compared to control mice. In contrast, β-actin immunoreactivity remained constant (Fig. 1A). Statistical analysis confirmed the significant decrease of APE/Ref-1 starting 1 h after I/R (O.D. of the APE/Ref-1; Nor., 11,563.8±287.7; 30 min to 24 h, 12,880.3±2039.2, 6711.3±2822.4, 4706.5±1986.1, 4226.5±2208.1 respectively). As shown in Fig. 1B, the number of AP sites per 100,000 nucleotides at each time point was counted and analyzed. At 30 min and 1 h after I/R, the number of AP sites was not noticeably increased, but rather was sustained at the same level as the normal control. However, the number of AP sites increased significantly from 4 to 24 h after I/R (AP sites, Nor., 1.82±1.72; 30 min, 3.54±1.11; 1 h, 4.24±0.81; 4 h, 13.84±1.72; 8 h, 24.24±0.91; 24 h, 26.67±1.11).

2.3. *Adenovirus-mediated gene expression*

Adenovirus particles were detected by double staining with type 5 hexon (green), one of the major structural components of the capsid protein, and PI (red) in the adenoviral vector-injected hemisphere, cortex, and striatum of mice, suggesting that the adenovirus was well distributed in target areas (Fig. 2A). After injection of Adv-APE/Ref-1, the amount of APE/Ref-1 was measured by western blot analysis of proteins extracted from the brains of mice (Fig. 2Ba, O.D. Normal, 300±23; day 2, 320±25; day 3, 680±45; day 4, 610±39; day 5, 250±35). APE/Ref-1 was increased at days 3 and 4 compared to the normal control group. However, the level of APE/Ref-1 in the control virus-treated group showed no difference to the normal control group (Fig. 2Bb). These results

Table 1 – Regional cerebral blood flow and physiological variables in mice.

Regional cerebral blood flow				
	n	10 min before occlusion (% if baseline)	10 min after occlusion (% if baseline)	10 min after reperfusion (% if baseline)
RCBF (%)	5	100±0	20.1±10.3	96.7±11.1
Physiological variables				
	n	Before/after administration of Adv-APE/Ref-1	Before/after administration of control virus	
Mean arterial blood pressure (mm Hg)	4	76.5±3.7/75.25±7.9	83.5±3.87/78.25±2.75	
$PaCo_2$ (mm Hg)	4	28.25±5.3/33.5±3.87	27.5±5.3/30.75±4.64	
PaO_2 (mm Hg)	4	154.25±11.87/160.75±7.76	165.25±5.05/165.5±9.29	
pH	4	7.28±0.08/7.4±0.01	7.26±0.06/7.35±0.05	
Rectal temperature (°C)	4	37.32±0.22/37.52±0.25	36.82±0.1/36.76±0.21	

mean±SD of each value.