BRAIN RESEARCH 1176 (2007) 143–150

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BRAIN RESEARCH

Research Report

Prevention of neuronal damage by calcium channel blockers with antioxidative effects after transient focal ischemia in rats

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ARTICLE INFO

Article history:
Accepted 11 July 2007
Available online 2 August 2007

Keywords:
Cerebral ischemia
Calcium channel blocker
Azelnidipine
Amlodipine
Oxidative stress

ABSTRACT

Background: Cerebral ischemia is a major leading cause of death and at the first place cause of disability all over the world. There are a lot of drugs that are in experimental stage for treatment of stroke. Among them are calcium channel blockers (CCBs) that have, in animal models, different effectiveness in healing of ischemic damage in brain. Mechanism of CCBs' action in cerebral ischemia is still unclear, but antioxidative property is supposed to be implicated. In the present study, we investigated antioxidative and neuroprotective properties of two CCBs, azelnidipine and amlodipine. *Methods:* Male Wistar Kyoto rats were subjected to 90 min of transient middle cerebral artery occlusion (MCAO) by a nylon thread. Animals were divided into 3 groups, vehicle, azelnidipine and amlodipine group. In the azelnidipine and amlodipine groups, rats were treated with azelnidipine (1 mg/kg) and amlodipine (1 mg/kg) by gastric gavage for 2 weeks before MCAO. Vehicle group was treated by solution of methyl cellulose for 2 weeks. Rats were killed 24 h after MCAO. Physiological parameters (mean arterial pressure, heart rate, body weight), infarct volume, brain edema index, cerebral blood flow (CBF), oxidative stress markers which are HEL, 4-HNE, AGE and 8-OHdG, and evidence of apoptosis by TUNEL, were investigated. *Results:* There were no significant differences among groups in mean arterial pressure, heart rate and body weight. Treatment with azelnidipine and amlodipine reduced infarct volume and brain edema. Azelnidipine treated group showed more marked reduction of infarct volume and cerebral edema than amlodipine group. There was no attenuation of CBF in CCBs groups. The number of HEL, 4-HNE, AGE and 8-OHdG positive cells were significantly decreased in the CCBs treated groups. These molecules were again fewer in the azelnidipine group than in the amlodipine group. In TUNEL staining, the numbers of positive cells was smaller in the CCBs treated groups, especially in the azelnidipine group. *Conclusions:* Pretreatment of azelnidipine and amlodipine had a neuroprotective effect in ischemic brain. Antioxidative property is one of the important profiles of CCBs that is implicated in brain protection.

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doi:10.1016/j.brainres.2007.07.038

1. Introduction

Since cerebral infarct is a major leading cause of death in the world and at the first place cause of disability, effective therapy or prevention of this event is of great importance. In the ischemic region, most cells have energy failure and undergo degeneration. It is difficult to rescue these cells unless successful cerebral blood flow (CBF) restoration was performed in acute stage. In the penumbra, on the other hand, the mechanism of cell death could be more complicated (Rossini et al., 2003). Not only energy failure, but also other modifying factors are quite important. It may be possible to rescue neuronal cells if appropriate pharmacological intervention is carried out because energy failure is milder in this region. Indeed, anti-apoptotic molecules, trophic factors, glutamate antagonists, sodium-channel blockers and free radical scavengers have reduced infarct volume by rescuing neuronal cells in the penumbral region.

Calcium channel blockers (CCBs) are widely used for hypertension (Opie and Schall, 2002). Clinical studies demonstrated their effectiveness in decreasing morbidity and mortality of vascular diseases such as stroke (Meairs et al., 2006), angina pectoris and hypertension (Kuramoto et al., 2003). On the other hand, vessels may not be the only target of CCBs. Since calcium influx is profoundly involved in neuronal cell death, CCBs should directly protect neurons under ischemia. Actually, there are many reports in which direct neuroprotective property of CCBs was demonstrated (Abe and Kogure, 1988; Mason, 2003; Sada and Saito, 2003).

Is there not any other mechanism of CCBs' neuroprotection? Several CCBs have dihydropyridine ring which can reduce oxidative stress (Park et al., 2006; Yao et al., 2000). We thus speculated that anti-oxidative property was implicated in CCBs' neuroprotection (Umemoto et al., 2004; Iwai et al., 2006). There are also in vitro studies that investigated anti-oxidative role of CCBs in different cell types (Shinomiya et al., 2004; Ma et al., 2006). In the present study, therefore, we used azelnidipine and amlodipine, both having dihydropyridine ring, and investigated their effects on oxidative damage and neuronal cell death in rat cerebral infarct model.

2. Results

Vehicle-, amlodipine- and azelnidipine-treated animals showed similar and no significant differences in mean arterial pressure (MAP), heart rate and body weight (Table 1).

Brain infarct volume of the vehicle-, amlodipine- and azelnidipine-treated rats were 345.7±14.2 mm^3, 306.8±24.5 mm^3 and 276.5±16.3 mm^3, respectively, showing a significant reduction by the drugs treatment and a stronger reduction by azelnidipine (Figs. 1A, B, $^*p<0.05$ vehicle vs. amlodipine and amlodipine vs. azelnidipine, $^{**}p<0.01$ vehicle vs. azelnidipine). Edema index was also significantly improved in the amlodipine- (28.5±0.6%) and azelnidipine-treated groups (24.0±1.6%), with more significance in the azelnidipine group, compared to the vehicle-treated group (31.2±1.4, Fig. 1C, $^*p<0.05$, $^{**}p<0.01$).

Occlusion of the middle cerebral artery resulted in a reduction of cerebral flow to approximately 20% of the baseline. After 5, 10 min of ischemia CBF stayed unchanged. Measurement of the relative cortical blood flow showed no significant differences among the CCBs groups and the vehicle group (Fig. 2).

Table 1 – Physiological parameters (MAP—mean arterial pressure, HR—heart rate and BW—body weight) in Wistar Kyoto rats

	Vehicle	Amlodipine	Azelnidipine
MAP (mm Hg)	98.5±7.7	94.6±5.2	93.0±6.7
Heart rate (/min)	363.9±21	349.2±27.9	344.7±15
Body weight (g)	264.6±9.1	270.2±11.4	266.8±4.7

There were no significant differences among the groups in MAP, HR and BW.

Fragmentation of cellular DNA was identified by TUNEL staining. The number of TUNEL positive cells (Fig. 3) in the peri-infarct area was reduced in the amlodipine and azelnidipine groups. Those in the vehicle, amlodipine and azelnidipine groups were 114.2±19.2, 71.1±13.8 and 61.1±10.2, respectively. Amlodipine or azelnidipine treatment significantly decreased DNA fragmentation ($^{**}p<0.01$ against vehicle group). In the azelnidipine group, the number of positive cells was smaller than those in the amlodipine group, but without statistical significance ($p=0.079$).

In the amlodipine- and azelnidipine-treated rats, the immunoreactivitiy for HEL, 4-HNE, AGE and 8-OHdG was less strong than that in the vehicle group (Fig. 4, left panels). In the azelnidipine-treated animals, the immunoreactivity was far less strong than that in the amlodipine-treated animals (Fig. 4). Quantitative analysis showed that the numbers of positive cells for HEL, 4-HNE, AGE and 8-OHdG were significantly smaller in the CCB groups ($^{**}p<0.01$) than in the vehicle group, with the azelnidipine more prominently than in the amlodipine group ($^*p<0.05$).

3. Discussion

Results of this study showed that treatment with CCBs reduced infarct volume, apoptosis and oxidative stress in the ischemic brain. This is the first report, to our knowledge, which demonstrated CCBs' anti-oxidative property in rat cerebral infarction model.

Why azelnidipine and amlodipine reduced oxidative stress? These L-type CCBs' anti-oxidative property may stem from their chemical structure; they contain aromatic ring, which can catch free radicals. Furthermore, the dihydropyridine ring in these CCBs is able to donate proton which should stabilize free electron (Masumoto et al., 1995). As they are lipophylic, they easily enter neuronal cells and should prevent lipid peroxidation (Mogi et al., 2006).

Another possibility is directly related with its effect to inhibit calcium influx. Although N- and P/Q-type calcium channels are implicated in calcium influx into cytoplasm, L-type calcium channel is more important for calcium-related cell damage. Previous experiments showed that azelnidipine prevented intracellular calcium accumulation and protected cells. Calcium overload, especially that of mitochondria (Tanaka et al., 2004; Kobayashi et al., 2003), generates free radicals (Cano-Abad et al., 2001; Yamanaka et al., 2003). CCBs in