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Research Report

Synergistic benefit of combined amlodipine plus atorvastatin on neuronal damage after stroke in Zucker metabolic rat

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ABSTRACT

Stroke is a major neurologic disorder and a leading cause of death in the world. We compared neuroprotective effects of single or combination therapy of amlodipine (AM) and atorvastatin (AT) in such a metabolic syndrome model Zucker rat after 90 min of transient middle cerebral artery occlusion (tMCAO). The animals were pretreated with vehicle, AM, AT, or the combination of AM plus AT for 28 days, and at 24 h of tMCAO, infarct volume and immunohistochemical analyses were performed. The combination of AM plus AT treatment decreased the infarct volume stronger than each single treatment with AM or AT. The numbers of positive cells of oxidative stress markers such as 8-hydroxy-2′-deoxyguanosin (8-OHdG), 4-hydroxy-2-nonenal (4-HNE), and advanced end glycation products (AGE) and inflammation markers such as tumor necrosis factor alpha (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) decreased dramatically in the combination-treated group compared with single AM- or AT-treated group. The present study showed that single AM or AT treatment showed neuroprotective effects both with antioxidative and anti-inflammatory mechanisms, but combination therapy of AM plus AT presented a further synergistic benefit in acute ischemic neural damages.

1. Introduction

Stroke is a major neurologic disorder and a leading cause of death in the world. Oxidative stress is strongly related to the pathophysiology of stroke (Abe et al., 1995; Hayashi et al., 1999), and inflammatory response is one of the first immune processes after injury relating after oxidative stress. Therefore, antioxidative and anti-inflammatory actions could be an important strategy in decreasing ischemic brain damage (Sun et al., 2002; Villegas et al., 2004).

The calcium channel blocker (CCB) AM is most commonly used for hypertensive patients in the world and does not only lower blood pressure but also directly protect neurons under ischemic damage (Opie and Schall, 2002; Lukic-Panin et al., 2007). Statin (3-hydroxy-3-methylglitaryl coenzyme A, HMG-CoA reductase inhibitor) is also widely used for lowering serum

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Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosin; 4-HNE, 4-hydroxy-2-nonenal; AGE, advanced end glycation products; TNF-α, tumor necrosis factor alpha; MCP-1, monocyte chemoattractant protein-1; MCA, middle cerebral artery; AM, amlodipine; AT, atorvastatin

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cholesterol level in the world (Farmer, 1998), among which AT has strong pleiotropic effects that decreased the risk of stroke and other cardio vascular events in patients with hyperlipidemia (Amarenco et al., 2006).

Zucker-fatty rat is a good model of obesity and metabolic syndrome with deficient leptin receptor (Vaziri et al., 2005), showing serum characters of insulin resistance, hypertriglyceridemia, and hyperlipidemia (HL) (Zucker, 1965; Zucker and Antoniades, 1972). Metabolic syndrome is the major risk factor for cardiovascular and ischemic stroke, needing single therapy not only for such as HT or HL but also for both diseases (Malik et al., 2004; Arenillas et al., 2009). Although there have been several reports of single treatment with AM (Yamagata et al., 2004; Lukic-Panin et al., 2007) or AT (Hayashi et al., 2005; Nagotani et al., 2005; Lee et al., 2008; Cui et al., 2010), their combination has not been investigated with respect to neuroprotection focusing on antioxidative and anti-inflammatory effects. In the present study, therefore, we compared neuroprotective effects of single or combination therapy of AM and AT in such a metabolic syndrome model Zucker rat after transient middle cerebral artery occlusion (tMCAO).

2. Results

2.1. *Infarct volume*

Compared with vehicle-treated controls (190.0±50.6 mm^3, $n=5$), quantitative analysis showed that AM-treated (146.7±27.8 mm^3, $n=5$, $^{*}p<0.05$) and AT-treated (124.1±13.6 mm^3, $n=5$, $^{*}p<0.05$) groups significantly reduced the infarct volume determined by CV staining (Fig. 1, upper pictures). Moreover, the combination of AM plus AT-treated group greatly decreased the infarct area (82.3±19.0 mm^3, $n=5$ $^{**}p<0.01$ vs vehicle group, $^{\#}p<0.05$ vs AM and AT groups), (Fig. 1, lower illustration).

2.2. *Physiological parameters in Zucker rat*

We monitored physiologic parameters, including regional cerebral blood flow during the experiments, and found no significant differences among the four experimental groups. Blood pressure was decreased in the amlodipine-treated group compared with other groups ($p<0.01$).

2.3. *Peroxidative markers*

Immunohistochemical analysis showed that no staining for 8-OHdG, 4-HNE, and AGE in the sham control brain (Fig. 2, sham) but that tMCAO induced strong staining of 8-OHdG, 4-HNE, AGE in nerve cells of the ischemic core (core) and the boundary penumbral (penumbra) zones (Fig. 2, vehicle). Compared with this vehicle group, single treatment with AM or AT significantly reduced the number of positively stained cells for these oxidative injury markers ($^{*}p<0.05$, $^{**}p<0.01$) in the ischemic core (Fig. 2, upper pictures) and the ischemic penumbra (Fig. 2, lower illustrations). Among the single treatment, treatment with AT decreased the number of positively stained cells compared with AM treatment both in the ischemic core and the ischemic penumbra ($^{+}p<0.05$, $^{++}p<0.01$ vs AM group). Combination of AM plus AT treatment showed a further reduction of the number of positively stained cells of 8-OHdG both in the ischemic core and the ischemic penumbra compared with AM or AT single treatment group ($^{\#}p<0.05$,

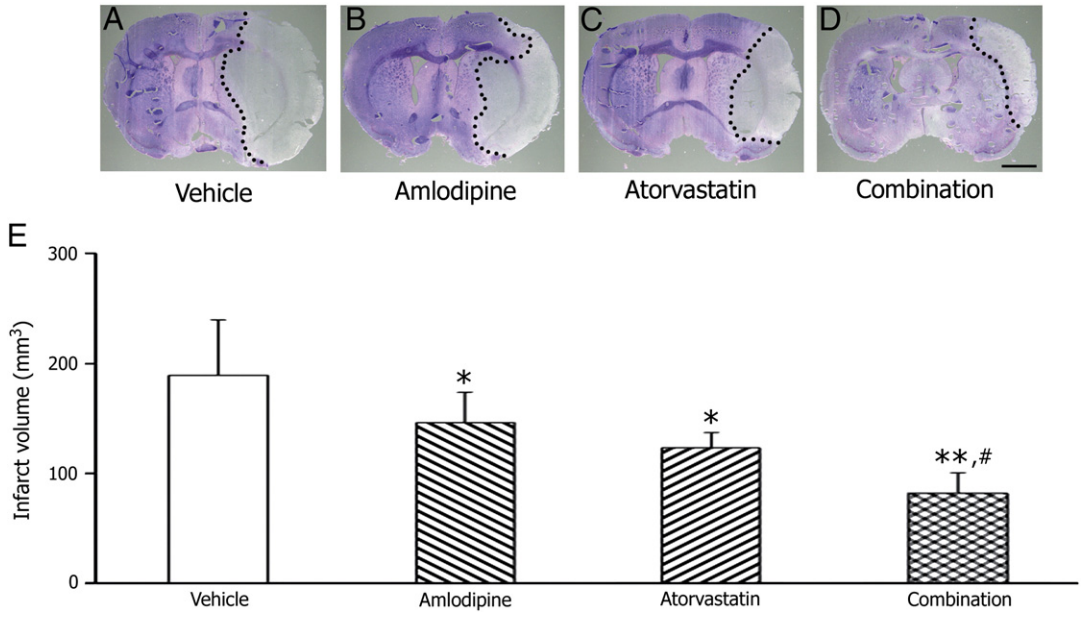

Fig. 1 – Brain infarct volume measured by cresyl violet staining. The sections at 24 h after tMCAO treated with vehicle (A), AM (B), AT (C), or AM plus AT combination (D). The AM, AT, and AM plus AT combination groups significantly reduced infarct volumes compared with the vehicle group ($^{*}p<0.05$, $^{**}p<0.01$), with a further reduction in the AM plus AT combination group compared with the single AM or AT treatment group ($^{\#}p<0.05$) (E). Scale bar=2 mm.