Labratory Research

Amlodipine inhibits matrix metalloproteinases expression and secretion in mouse macrophage

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Objective To investigate whether the calcium channel blocker amlodipine could inhibit macrophage matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) expression and secretion. Methods Peritoneal macrophages were isolated from BALB/C mice and incubated with low (5 µg/L), middle (15 µg/L) and high (30 µg/L) concentrations of amlodipine, or in the medium alone (controls) for 24 hours, and the expression and secretion of MMP-2 and MMP-9 of the cells were analyzed by RT-PCR and gelatin zymography. Results Compared with controls, amlodipine at low concentration had no significant effects on the expression and secretion of either MMP-2 and MMP-9 (P>0.05) at middle concentration it could inhibited MMP-2 and MMP-9 expressions completely and significantly reduced the secretion of MMP-9 (P<0.05); but it had no effect on the secretion of MMP-2. At high concentration it also inhibited MMP-2 and MMP-9 expression completely. Conclusion Amlodipine at 15 µg/L inhibited the expression of MMP-2 and MMP-9 and reduced the secretion of MMP-9, suggesting that amlodipine may stabilize atherosclerotic plaque.

Key Words amlodipine; macrophage; matrix metalloproteinase; reverse transcriptase polymerase chain reaction; gelatin zymography.

The mechanisms of atherosclerotic plaque rupture and factors affecting plaque stability remains to be fully understood. It is known that plaque disruption with superimposed thrombosis results in acute coronary syndromes (unstable angina, myocardial infarction, and sudden death). It was estimated that 90% of acute myocardial infarction results from plaque disruption with superimposed thrombosis. Many studies have documented that matrix metalloproteins (MMP), especially MMP-2 and MMP-9, produced by macrophages, might play key roles in extracellular matrix degradation and plaque rupture.

As one of the long-acting dihydropyridine calcium antagonists, amlodipine is widely used in the treatment of hypertension. Recent studies also showed that amlodipine can reverse the left ventricular hypertrophy as effectively as angiotension II converting enzyme inhibitors (ACEIs). Like other calcium antagonists, it is also used, in combination with β-blockers, in the treatment of both stable and unstable coronary artery disease (CAD). However, to our knowledge, there has been no study to assess amlodipine’s effect on plaque stability and its role in the prevention of acute coronary syndromes is not clear. In this study, we explored the effects of amlodipine on the expression and secretion of MMP in mice peritoneal macrophage and evaluated its potential role in stabilizing atherosclerotic plaques.

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Materials and methods

Animals and materials
Pathogen-free BALB/C mice (8 to 12 weeks old, female) were obtained from Harbin Medical University Animal Center. RPMI 1640 medium was purchased from GIBCO (USA), Trizol Reagent from BIO. TECH. COMPANY (USA), RT-PCR kit from Clontech (USA), Gelatin from Sigma (USA). Amlodipine was provided by Pfizer Company (Dalian, China).

Cell isolation and cell culture
Mice peritoneal macrophages were obtained by peritoneal lavage using 8 ml cold sterile saline. A total of 2 × 10³ to 5 x 10³ cells were harvested from each mouse. Cells were collected by centrifugation, washed, and resuspended twice in RPMI 1640 medium supplemented with 4 mmol glutamine, 100U/ml penicillin, 100 mg/ml streptomycin. The cells were incubated for 2 h at 37 °C, then gently rinsed to remove non-adherent cells. Collected in this way, 98% of the adherent cells were macrophages. The cells were incubated with low (5 µg/L), middle (15 µg/L) or high (30 µg/L) concentrations of amlodipine for 24 hours at 37 °C in a CO2 incubator. For controls, macrophages were incubated in the medium without amlodipine.

RNA preparation and RT-PCR
Cells were washed with ice-cold PBS, and total RNA
was isolated with Trizol according to the manufacturer’s instructions. For reverse transcriptase (RT)-PCR, RNA was treated with RNase-free DNase, and cDNA was synthesized using Moloney murine leukemia virus RT (Invitrogen Life Technologies, Carlsbad, CA). Each cDNA (about 2.5%) was subjected to 22-25 cycles of PCR under conditions that resulted in a single specific amplification product of the correct size: 30 sec denaturation at 94°C, 1 min annealing at 55°C, and 45 sec extension at 72°C in a GeneAmp 9600 thermal cycler (Perkin-Elmer, Foster City, CA). Oligonucleotide primers used were as follows: MMP-2, sense 5'-GAGTTGGCAGTGCATACCT-3' and antisense 5'-GCCGTCCTTC TCAAGTTGAC-3', 665bp; MMP-9, sense 5'-AGTTTGGTGTCGCGGA GC-3' and antisense 5'-TACATGAGCGCTTCCGGCAC-3', 750bp. To adjust the amount of transcribed cDNA, β-actin was selected as an internal control. The primer sequences for β-actin were sense 5'-GACGATATCGCCGCGCTCGCC-3' and antisense 5'-GCCACGCTCC AGACGCAGGATG-3', 314bp. 10μl of PCR products were separated on a 1.5% agarose gel containing ethidium bromide.

**Results**

**Effect of Amlodipine of various concentrations on the mRNA expression of MMP-2 and MMP-9 in mice macrophages:**

Mice macrophages were cultured with amlodipine of various concentrations for 24h. When the amlodipine concentration was 5μg/L, it had little effect on the expression of MMP-2 and MMP-9 (P>0.05) of the cells. When the concentration was 15μg/L or 30μg/L, amlodipine stopped the expression of MMP-2 and MMP-9 completely, there was no production of MMP-2 and MMP-9 found in RT-PCR produce (Fig.1 and Fig. 2).

**Effects of amlodipine of various concentrations on the secretion of MMP-2 and MMP-9 in mice macrophages:**

Mice macrophages were cultured with amlodipine of various concentrations for 24h. When the concentration was 5μg/L, amlodipine had little effect on the secretion of MMP-2 and MMP-9 (P>0.05). When the concentration was 15μg/L, the secretion of MMP-9 was inhibited obviously (P<0.05), but MMP-2 was inhibited just lightly and had no statistical significance (P>0.05). When the drug concentration was 30μg/L, the quantity of MMP-9 and MMP-2 increased remarkably, but they may come from the broken dead cells (Fig. 3 and Table 1).
metalloproteinase (MMPs). Therefore, inhibiting the expression and secretion of MMPs has been suggested as a treatment target to decrease atherosclerotic plaques rupture and reduce the incidence of cardiovascular events. To date, no drugs have been used to inhibit the expression and secretion of MMPs in clinic. Our study showed that at the concentration of 5\(\mu\)g/L (at which clinical study showed can lower blood pressure by about 10 mmHg), amlodipine had little effect on the expression and secretion of MMP-2 and MMP-9 in mice peritoneal macrophages. When the concentration was 15\(\mu\)g/L (at which average can lower blood pressure by about 20 mmHg), the expression of MMP-2 and MMP-9 in mice peritoneal macrophages were completely inhibited, the mRNA production could not be found in RT-PCR analysis. So, at the clinically therapeutic concentration, amlodipine might play some roles in the inhibition of the expression of matrix metalloproteinase. When the drug concentration was 30\(\mu\)g/L (at this concentration, amlodipine can lower blood pressure by about 30 mmHg), the expressions of MMP-9 and MMP-2 were inhibited completely, and dead macrophages could be seen in the media supernatants. Dead macrophages broke and released the MMPs, so the increased activity of MMP-2 and MMP-9 was observed. Therefore, from the clinical point of view, it would be helpful to find an optimal therapeutic concentration of amlodipine, at which amlodipine not only could lower blood pressure effectively, but can also inhibit the expression of MMP-2 and MMP-9 and stabilize plaque.

Macrophages derived foam cells in atherosclerotic plaque are one of the main sources of MMPs. We do not know whether amlodipine could also inhibit the MMPs expression in these cells. Further studies should be undertaken to evaluate amlodipine’s role in stabilizing atherosclerotic plaque, and therefore in reducing cardiovascular events.

### References