Effects of Combined Blockade of AT_1 Receptor and HMG-CoA Reductase on Left Ventricular Remodeling in Infarcted Rats

Running title: Pravastatin, Olmesartan and Remodeling

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Abstract

***Background*—**Cardiac remodeling was associated with myocardial hypertrophy and left ventricular (LV) dilation following myocardial infarction. These changes in LV geometry contribute to the development of depressed cardiac performance, arrhythmias and sudden cardiac death. Accumulating evidence indicates that angiotensin II plays a key role in the pathophysiology of myocardial hypertrophy after myocardial infarction. Angiotensin receptor blockers (ARBs) favorably modulate extracellular signal-regulated protein kinase to elicit attenuated cardiac hypertrophy. There is considerable evidence that electrophysiological changes were associated with the hypertrophied myocardium. Hypertrophied myocardium has been shown to generate arrhythmias more readily than normal tissue. Agents with the regression of ventricular hypertrophy have been shown to decrease the susceptibility of ventricular arrhythmias. ARBs have been shown to provide the survival benefit by regression of ventricular hypertrophy and reduction of arrhythmic death in animals and in patients. However the regression of hypertrophy is suboptimal by administering ARBs, and thus it may not sufficiently antagonize hypertrophy progression and thereby reduce the risk of major cardiac events. It is of interest to identify drugs other than ARBs to reduce cardiac hypertrophy. We have previously demonstrated that pravastatin can attenuate ventricular hypertrophy by inhibiting the endothelin-1 expression separate from their cholesterol-lowering actions in hyperlipidemic patients and in normolipidemic animals. The usefulness of ARB and statins after infarction has been demonstrated to attenuate cardiomyocyte hypertrophy individually. However, whether their coadministration has an additive effect on ventricular remodeling and the pharmacological mechanisms underlying the benefit of the combination therapy remain poorly understood. Thus, we assessed whether pravastatin provides an additive effect on prevention of ventricular hypertrophy by inhibiting endothelin-1 expression after myocardial infarction in rats cotreated with olmesartan, a nonpeptide ARB.

***Methods and Results*—**After ligation of the left anterior descending artery, rats were randomized to both, 1, or neither of the angiotensin receptor antagonist olmesartan (0.01, 0.1, 1, and 2 mg/kg per day) and HMG-CoA reductase inhibitor pravastatin (5 mg/kg/day) for four weeks. Each drug, when given alone, decreased cardiomyocyte sizes isolated by enzymatic dissociation at the border zone compared with vehicles. However, compared with either drug alone, combined olmesartan and pravastatin prevent cardiomyocyte hypertrophy to a larger extent (Table 1), which was further confirmed by downregulation of left ventricular atrial natriuretic peptide mRNA (Figure 1). The Pearson linear regression models showed that there was a significant correlation between the reduction of cardiomyocyte hypertrophy and the dose of olmesartan (0.01, 0.1, 1 and 2 mg/kg) in the combined therapy (reduction in cardiomyocyte hypertrophy (%) = 6.6*the dose of olmesartan (mg/kg per day) + 19.1, r = 0.88, P=0.046).

The myocardial endothelin-1 levels at the border zone were 6.5-fold higher (P <0.0001) in the vehicle group compared with the sham group, which can be inhibited after pravastatin administration. Combination treatment significantly attenuated cardiomyocyte hypertrophy in a dose-dependent manner although tissue endothelin-1 levels remained stable in combination groups of different olmesartan doses. Immunohistochemical analysis confirmed the changes of endiothelin-1. Measurements of arrhythmic score mirrored those of cardiomyocyte hypertrophy.

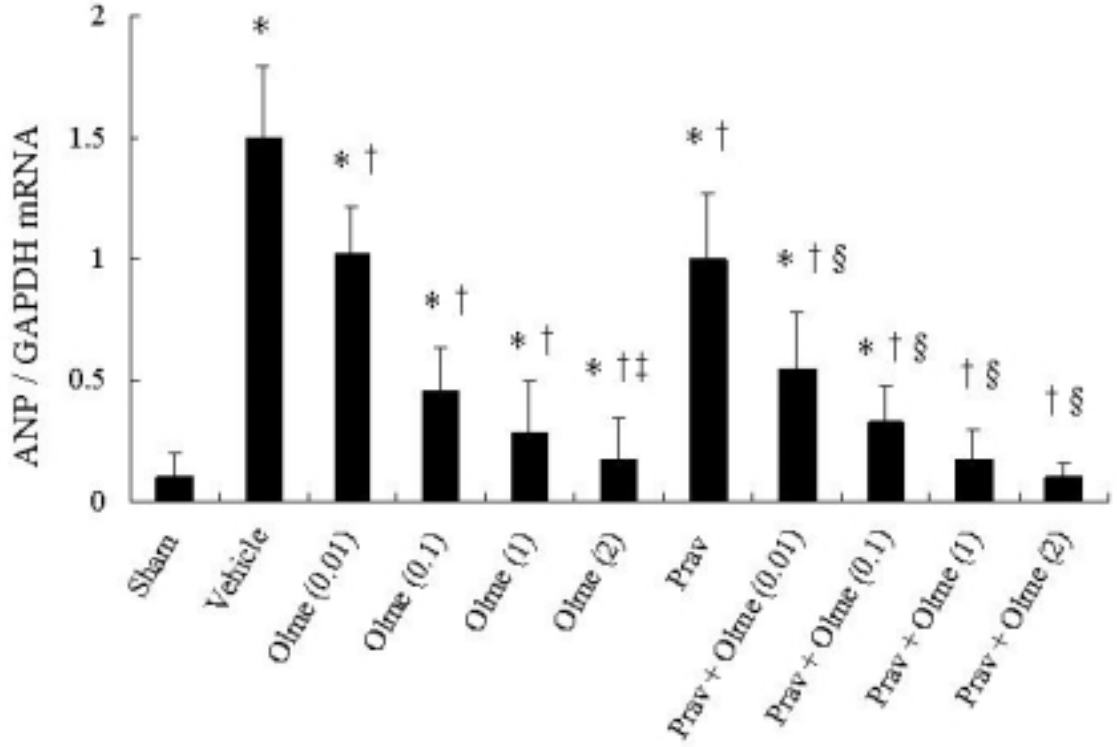
Conclusions—Combined therapy is more effective in reducing ventricular remodeling than pravastatin and olmesartan alone in rats after myocardial infarction through different mechanisms. Pravastatin administration provided favorable ventricular remodeling probably through decreased tissue ET-1 level. In contrast, olmesartan-related attenuated cardiomyocyte hypertrophy is independent of ET-1 pathway.

Table 1. Characteristics of isolated cardiomyocytes at the border zone.

Parameters	Sham	Vehicle	Olme (0.01 mg/kg)	Olme (0.1 mg/kg)	Olme (1 mg/kg)	Olme (2 mg/kg)	Prav	Prav + Olme (0.01 mg/kg)	Prava + Olme (0.1 mg/kg)	Prav + Olme (1 mg/kg)	Prav + Olme (2 mg/kg)
Number of animals	4	4	3	3	4	4	4	4	4	3	4
Myocyte length, μm	135 ± 10	188 ± 25*	152 ± 23*†	154 ± 18*†	149 ± 17*†	137 ± 26†	154 ± 25*†	134 ± 23†‡	134 ± 18†‡§	125 ± 24†‡§	125 ± 22†‡§
Myocyte width, μm	21 ± 4	23 ± 3*	21 ± 5†	21 ± 3†	21 ± 4†	20 ± 2*	21 ± 3†	21 ± 3†	20 ± 2†	21 ± 2†	20 ± 2†
Measured myocyte areas, μm^2	2847 ± 257	4527 ±241*	3784 ± 346*†	3462 ± 312*†	3425 ± 276*†	3045 ± 377*†‖	3502 ± 266*†	3020 ± 368†‡§	2951 ± 294†‡§	2895 ± 481†‡§	2774 ± 202†‡§¶

Values are mean ± SD. Olme, olmesartan; Prav, pravastatin. *P<0.05 compared with the sham-operated group; †P<0.05 compared with the vehicle group; ‡P<0.05 compared with the olmesartan group alone; §P<0.05 compared with the Pravastatin group; ‖P<0.05 compared with Olmesartan (0.01 mg/kg per day); ¶P<0.05 compared with Pravastatin + Olmesartan (0.01 mg/kg per day).

Figure 1. Left ventricular ANP mRNA measured by competitive RT-PCR at the border zone in rats of the sham, the vehicle-treated rats, and the infarcted rats treated with olmesartan (0.01 mg/kg), olmesartan (0.1 mg/kg), olmesartan (1 mg/kg), olmesartan (2 mg/kg), pravastatin (5 mg/kg), pravastatin + olmesartan (0.01 mg/kg), pravastatin + olmesartan (0.1 mg/kg), pravastatin + olmesartan (1 mg/kg), and pravastatin + olmesartan (2 mg/kg). Each mRNA was corrected for an mRNA level of GAPDH. Each column and bar represents mean ± SD. **P*<0.05 compared with the sham-operated group. †*P*<0.05 compared with the vehicle group; ‡*P*<0.05 compared with olmesartan (0.01 mg/kg per ay); §*P*<0.05 compared with the same dose of olmesartan alone. Olme, olmesartan; Prav, pravastatin.


ANP / GAPDH mRNA
2
1.5
1
0.5
0
* †
* †
* †
* †
* †‡
* †
* † §
* † §
† §
† §
Sham
Vehicle
Olme (0.01)
Olme (0.1)
Olme (1)
Olme (2)
Prav
Prav + Olme (0.01)
Prav + Olme (0.1)
Prav + Olme (1)
Prav + Olme (2)