EFFECT OF RALOXIFENE ON MATRIX METALLOPROTEINASES EXPRESSION IN CULTURED MACROPHAGES AND SMOOTH MUSCLE CELLS

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The composition of the atherosclerotic lesions, more than their dimension, is the most important determinant of cardiovascular disease such as miocardial infarction, stroke, suddently death. The extension of lipid core and the integrity of the fibrous cap determine the stability and trombogenicity of atherosclerotic lesion. Activated macrophages within the arterial wall secrete matrix-degrading metalloproteinases (MMPs) that weaken the atherosclerotic plaque and contribute to its fissuration, leading to atherosclerosis complications. Raloxifene is a selective estrogen receptor modulator in clinical experimentation for primary and secondary prevention of cardiovascular disease in postmenopausal women. Raloxifene is an agonist of estrogen receptors in the cardiovascular system, acting both on lipid metabolism and vascular wall. We have recently shown that raloxifene reduces macrophages accumulation and MMPs expression in carotid lesions of ovariectomized hypercholesterolemic female rabbits subjected to perivascular carotid placement collar. Collar-induced lesions showed conspicuous macrophage infiltration, and elevated expression of MMP-1, MMP-3, and MMP-9. Raloxifene significantly reduced RAM-11 positive macrophage accumulation (approximately –60%, $p<0.05$), and the amount of lesion area positive to MMP-3 (-34%, $p<0.001$) and MMP-9 (-73%, $p<0.001$). The reduction of MMP-1 (-23%) was not statistically different. To elucidate its mechanism of action, we have evaluated the effect of raloxifene on the *in vitro* expression of MMP-9 in murine peritoneal macrophages and of MMP-2 in smooth muscle cells (SMC) obtained from the carotids of rabbits that underwent a perivascular manipulation. Cells were incubated for 24 hours with increasing concentrations of raloxifene, and MMPs' gelatinolytic activity was measured in the conditioned media by gelatin zymography. In murine macrophages raloxifene treatment reduced in a concentration-dependent manner (0.1-10 μM) MMP-9 gelatinolytic potential induced by TNF-α up to 60% ($P<0.01$), without affecting MMP-9 activation. In these cells, raloxifene (5 μM) reduced by 64% the MMP-9 promoter-driven transcription of the luciferase reporter gene. Next, we measured the effect of raloxifene on MMP-2 activity in rabbit SMC derived from collared carotid artery. When added to the cells, raloxifene (0.1-5 μM) inhibited up to 40% MMP-2 gelatinolytic activity ($P<0.01$). This effect was due to a reduction of the amount of MMP-2 released by the cells. In the same experimental conditions, raloxifene was ineffective in SMC obtained from the contralateral artery.
Altogether, our results suggest that raloxifene is able to directly inhibit MMP-9 and MMP-2 gelatinolytic potential in cells involved in atherosclerotic plaque formation.