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Role of annexin II in estrogen-induced macrophage matrix metalloproteinase-9 activity: The modulating effect of statins

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Abstract

Annexin II (ANXII) is a receptor for tissue plasminogen activator and plasminogen for the conversion to plasmin, which, in turn, induces metalloproteinase-9 (MMP-9). 17β-Estradiol (E_2) is reported to decrease plasminogen activity inhibitor-1 and increase plasmin and matrix metalloproteinase activity. However, the combined effects of estrogen and statins on macrophage MMP-9 activity and ANXII expression remain unclear. Treatment of J774A.1 macrophages with 1.0–100 nM of E_2 for 24 h increased both MMP-9 activity and ANXII expression in a dose-dependent manner ($p < 0.05$). Preincubation with EGTA (10 mM) released ANXII from the cell membrane and inhibited the E_2-mediated MMP-9 activity as did incubation of macrophages with anti-annexin IgG. In the presence or absence of E_2 (5 nM), simvastatin treatment in the range of 0.1–5.0 μM significantly reduced macrophage MMP-9 enzymatic activity ($p < 0.005$) in a dose-dependent manner. In the presence or absence of E_2, simvastatin also decreased ANXII expression ($p < 0.05$). These findings indicate that ANXII plays a central role in modulating the enzymatic activity of MMP-9 in response to E_2 and that E_2-mediated ANXII expression and MMP-9 activity can be prevented by simvastatin. Prevention of E_2-mediated activation of MMP-9 by simvastatin suggests that concurrent statin use may account for early event risk of myocardial infarction seen with hormone therapy in recent clinical trials.

Keywords: Estrogen; Statins; Matrix metalloproteinases; Annexin II; Macrophages

1. Introduction

Two recent randomized double-blind, placebo-controlled trials have indicated a potential early risk of myocardial infarction within the first year of hormone therapy relative to placebo [1,2]. This observation is unique and not previously reported with any other therapy investigated for reducing coronary events. Although the pathophysiology underlying this observation is unclear, the immediacy of the ischemic events suggests destabilization of atherosclerotic plaques. This speculation is underscored by the lack of early risk of myocardial infarction with hormone therapy in those women who were concurrently using statins [3]. The protective effect of statins is consistent with the current paradigm that atheroprotective agents increase plaque stability.

An adverse effect of hormones on plaque stability is mechanistically supported by the relationship between endogenous estrogen levels and matrix metalloproteinase (MMP) expression. Endometrial tissue MMP expression and activity increase during the follicular estrogen phase of the menstrual cycle and facilitate sloughing of the endometrium. Exogenous administration of oral estrogen also increases plasma levels of MMPs [4]. These data indicate both a systemic and tissue specific effect of estrogen on MMP expression and activity. Expression of MMPs in an activated form within atherosclerotic plaques are significantly increased in

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the vulnerable regions of atherosclerotic plaques [5]. Recent studies indicate that estrogen activates MMP expression and activity in vascular cells through a rapid signaling pathway involving tyrosine kinases [6]. These findings suggest that adverse effects of estrogen on vascular tissues may include MMP activation as a mediator of plaque instability.

Annexin II (ANXII) plays an important role in the pathophysiological action of macrophages during the inflammatory process [7]. ANXII serves as an activator of MMPs by facilitating the interaction between t-PA and plasminogen with subsequent activation of plasmin [8]. Plasmin serves as a physiological activator which, in turn, converts pro-MMP-9 to active MMP-9 [9]. However, it remains unknown whether E_2-mediated increase in MMP activity requires the expression of ANXII in macrophages.

The effect of statins on macrophage MMP-9 have been reported [10,11], whereas the combined effect of estrogen and statins has not been previously described. In this study, we used an in vitro macrophage cell model to examine the effect of estrogen and statin and the role of ANXII on MMP activity. To parallel recent clinical observations, we hypothesized that macrophage MMP activity would be increased with 17β-estradiol (E_2) treatment and that the effect of E_2 on macrophage MMP activity would be reduced in the presence of statin. We also hypothesized that ANXII plays an important role in mediating the action of estrogen and statin on the expression of MMP-9.

2. Methods

2.1. Chemical and reagents

The following reagents were obtained from Sigma Chemical Co. (St. Louis, MO): 17β-estradiol, ethylenedinitro tetraacetic acid, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, Tris–HCl, glycerol, bromophenol blue, sodium dodecyl sulfate, Triton X-100, *p*-aminophenylmercuric acetate. Buffers, media, and cell culture supplies were obtained from Life Science (New York, NY), fetal bovine and human male serum from Omega Scientific (Van Nuys, CA). Anti-annexin II IgG and non-immune control rabbit IgG were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Simvastatin was provided gratis from Merck.

2.2. Cell culture

J774A.1 macrophages were purchased from ATCC (Manassas, VA). Cells were passaged using a 1:3 split ratio, allowed to grow to confluency and transferred by scrapping. DMEM containing 10% fetal bovine serum and 50 mg/ml gentamicin was used as the culture medium. Cultures were maintained at 37 °C in a humidified incubator (5% CO_2, 95% air). Experiments were performed when cells were about 90% confluent. Three days prior to the experiment, cells were split in DMEM phenol red free media containing 10% human male serum and were grown to approximately 90% confluency, followed by incubation for the indicated intervals in the absence or presence of E_2 in phenol red free and serum free media for 24 h.

2.3. Zymographic analysis of pro-MMP-9 activity

MMP-9 enzyme in the conditioned media was assayed with gelatin zymography as previously described [12]. Each aliquot of sample which was normalized based in cell numbers (4×10^5) was mixed with equal volumes of non-reducing lysis buffer (0.25 M Tris–HCl, pH 6.5; 20% glycerol; 2% SDS and 10 mg/ml bromophenol blue). After 30 min incubation at room temperature, the samples were loaded onto a 10% SDS polyacrylamide gel containing 1 mg/ml of gelatin as a substrate (BioRad Ready Gel, CA). After electrophoresis, gels were incubated in 2.5% Triton X-100 for 30 min with two changes of solution and incubated overnight with the substrate buffer (50 mM Tris, pH 8.0; 50 mM NaCl and 10 mM $CaCl_2$) at 37 °C. The gels were stained with 0.1% Coomassie Brilliant Blue and destained with 10% acetic acid solution. The gelatinolytic activity was measured by using the NIH Scion Image software program after the gels were scanned.

2.4. Western blot analysis

The level of annexin II (ANXII) was determined by Western blot analysis. After incubation with the various compounds, cells were scraped from the wells, washed with PBS, and the cell pellet subjected to protein analysis prior to blotting. The cell lysate was mixed with 5× Laemmli sample loading buffer, heated for 3 min at 100 °C and separated electrophoretically on 10% or 12% SDS-PAGE (BioRad Mini protein system, BioRad, CA). Proteins were transferred to Immobillon P membranes (Millipore Corporation, MA) at 100 A for 1.5 h. The membrane was blotted with 5% non-fat dry milk in TBS with Tween-20 for 45 min and incubated with annexin II antibody according to the manufacturer's guidelines (Santa Cruz Biotechnology, CA and Oncogene Research Product, MA). Chemiluminescence detection was used to visualize bands of interest (Pierce, IL). Photographic films were scanned by an imaging densitometer and quantified using NIH Scion Image software program.

2.5. Statistical analysis

All results are expressed as mean and standard errors determined from atleast five independent experiments with all measurements performed in duplicate. Determinations of statistical significance between various treatment groups were made using the paired two-tailed Student's *t*-test. Comparisons of multiple mean values were made by one-way analysis of variance (ANOVA). *p*-Values of <0.05 were considered statistically significant.