Curcumin Blocks HIV Protease Inhibitor Ritonavir-Induced Vascular Dysfunction in Porcine Coronary Arteries

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BACKGROUND: HIV protease inhibitor ritonavir (RTV) is associated with many cardiovascular complications and causes vascular dysfunction through oxidative stress. In the present study, we determined the effects of RTV and curcumin (a pigment derived from turmeric) on porcine coronary arteries.

STUDY DESIGN: Artery rings were incubated with 15 μM concentration of RTV and curcumin (5 or 10 μM) for 24 hours. Vasomotor function was studied with a myograph tension system. Endothelial nitric oxide synthase (eNOS) mRNA and protein levels were studied using real-time polymerase chain reaction, Western blot, and immunohistochemistry. Nitric oxide was measured using Griess assay. Superoxide anion levels were determined by lucigenin enhanced chemiluminescence.

RESULTS: RTV considerably reduced vessel contraction by 71%, endothelium-dependent relaxation by 59%, and endothelium-independent relaxation by 52%, as compared with controls. Curcumin effectively blocked RTV-induced vasomotor dysfunction. RTV-treated arteries showed substantial reductions of eNOS mRNA by 77%, eNOS protein by 72%, and nitric oxide release by 37% as compared with controls. The RTV plus curcumin-treated vessels showed substantial increases of eNOS and nitric oxide levels as compared with the RTV-treated vessels. Finally, there was a 47% increase of superoxide anion production in the RTV-treated vessels as compared with controls. Again, curcumin effectively reversed this effect of RTV.

CONCLUSIONS: HIV protease inhibitor RTV impairs vasomotor functions, reduces eNOS expression and nitric oxide release, and increases oxidative stress in porcine coronary arteries. Curcumin effectively blocks these detrimental effects of RTV. (J Am Coll Surg 2005;200:820–830. © 2005 by the American College of Surgeons)

Given the success of HIV protease inhibitors (PIs) in improving the survival of HIV-infected patients, long-term adverse events become a serious concern.[1] In initial reports, patients as young as 26 years of age using HIV PIs were associated with advanced atherosclerotic lesions. Additional investigations revealed an association between HIV PIs and several known risk factors for cardiovascular disease, including hyperlipidemia, lipodystrophy, and insulin resistance.[2,3] Although elevated blood lipid levels can account for the increase in atherosclerotic vascular disease associated with use of HIV PIs, there can also be some direct effects of these drugs on arterial walls. In a recent clinical cross-sectional study of HIV patients treated with or without PIs, there was a correlation between use of PIs and endothelial dysfunction.[4] The molecular mechanisms of these effects have not been thoroughly investigated.

Curcumin is a major active component of food-flavoring turmeric (*Curcuma longa*). It has been used for centuries in indigenous medicines for treatment of a variety of inflammatory conditions and other diseases.[5] It is shining as an antioxidant, for example, and for its

Competing Interests Declared: None.

This work is partially supported by research grants from the National Institutes of Health (Chen: R01 HL61943, R01 HL60135, R01 HL65916, R01 HL72716, and R01 EB-002436; Yao: R21 AI 49116 and R01 DE15543; Lumsden: R01 HL75824; and Lin: K08 HL076345).

Presented at the American College of Surgeons 90th Annual Clinical Congress, Surgical Forum, New Orleans, LA, October 2004.

Received December 2, 2004; Revised February 11, 2005; Accepted February 21, 2005.

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ISSN 1072-7515/05/$30.00
doi:10.1016/j.jamcollsurg.2005.02.030

Abbreviations and Acronyms

DHE = dihydroethidium
DMEM = Dulbecco's modified Eagle's medium
eNOS = endothelial nitric oxide synthase
NO = nitric oxide
O_2^- = superoxide anions
PCR = polymerase chain reaction
PI = protease inhibitors
RLU = relative light units
RTV = ritonavir

beneficial effects in prohibiting tumors, for its antiinflammatory properties, and even for its ability to retard some of the progress of the virus that causes AIDS. Recently, several studies have shown that curcumin is a potent inhibitor of tumor initiation and proliferation in vivo.[6-8] Besides its anticancer properties, studies have shown that relatively low concentrations of curcumin have remarkable antiinflammatory and antioxidant effects.[9-13]

My colleagues and I previously reported that ritonavir (RTV), one of the five clinically used PIs, can cause endothelial injury in human endothelial cells[14] and in porcine carotid arteries.[15] This damage is mostly induced by a remarkable increase of superoxide anion production. In this study, we hypothesized that curcumin can block RTV-induced vascular injury. Porcine coronary arteries were used to study the effect of RTV and curcumin on vasomotor functions, endothelial nitric oxide synthase (eNOS) expression, nitric oxide (NO) release, and superoxide anion production. Data from this study suggest a new therapeutic strategy to control HIV PI-associated cardiovascular complications.

METHODS

Chemicals and reagents

U46619 (9,11-Dideoxy-11α,9α-epoxymethanoprostaglandin F2α), bradykinin, sodium nitroprusside (SNP), curcumin, Tris-buffered saline solution, PBS solution, β-actin monoclonal antibody, Triagent kit, and Tween 20 were obtained from Sigma Chemical Co. Lucigenin and dihydroethidium (DHE) were obtained from Molecular Probes. Dulbecco's modified Eagle's medium (DMEM) was obtained from Life Technologies, Inc. Antibiotic-antimycotic solution was obtained from Mediatech Inc. iScript cDNA Synthesis Kit, iQ SYBR Green SuperMix Kit, protein assay Kit, nonfat dried milk, and precast polyacrylamide gels were obtained from Bio-Rad laboratories. Antibody against human eNOS was obtained from BD Transduction Laboratories. Horseradish peroxidase-conjugated goat antimouse secondary antibodies and the enhanced chemiluminescence kit were obtained from Amersham Life Sciences. Normal horse serum, biotinylated horse antimouse IgG, avidin-biotin complex kit, and diaminobenzadine were obtained from Vector Laboratories. RTV was obtained from AIDS research and Reference Reagent Program (NIH). Primers were obtained from Sigma Genosys.

Tissue harvest and culture

Fresh porcine hearts were harvested from young adult farm pigs (6 to 8 months old) at a local slaughterhouse, placed into a container filled with cold PBS solution, and immediately transferred to the laboratory. The pig's right coronary artery was carefully dissected. Vessels were cleaned of fat and connective tissue and cut into multiple 5-mm rings, which were then incubated in DMEM with 15 μM RTV alone or together with curcumin (5 or 10 μM) at 37°C and 5% CO_2 in a cell culture incubator for 24 hours. DMEM with 0.1% dimethylsulfoxide (v/v, solvents for RTV and curcumin) was used as control. One percent of antibiotic-antimycotic solution was added into the culture medium. The concentration of 15 μM RTV used in this study is similar to the maximal plasma concentration of patients who receive regular RTV therapy.[16]

Myograph analysis

The myograph system used in our laboratory has been described previously.[17,18] After cultured in the medium for 24 hours, rings were suspended between the wires of the organ bath myograph chamber (Danish Myo Technology Organ Bath 700 MO) in 6 mL Kreb's solution, maintained at 37°C and oxygenated with pure oxygen gas. Rings were slowly subjected stepwise to a predetermined optimal tension of 30 mN and allowed to equilibrate for at least 30 minutes. After equilibration, each ring was precontracted with 20 μL thromboxane A2 analogue U46619 (10^{-7} M). After 60 to 90 minutes of contraction, a relaxation dose-response curve was generated by adding 60 μL of five cumulative doses of the endothelium-dependent vasodilator bradykinin (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M) every 3 minutes. In addition, 60 μL SNP (10^{-6} M) was added into the organ bath and endothelium-independent vasorelax-