Enhancement of Cardio-Protective Effects and Attenuation of Adverse Effects of Female Sex Hormones on Cultured Human Vascular Smooth Muscle Cells by a Combination of Ascorbic Acid, Lysine, Proline, Arginine, Cysteine and Epigallocatechin Gallate (2003)

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Abstract

Introduction:
Estradiol has been shown to exert protective effects on the cardiovascular system. However, hormone replacement therapy has produced only limited benefits in clinical trials and, due to adverse effects, has recently become a controversial issue. Combined supplementation with ascorbic acid and lysine has been proposed as a preventive measure to atherosclerosis development. Furthermore, a worldwide search for the most cardio-protective plant-derived compounds, has suggested a number of possible candidates.

Objective:
These data prompted us to investigate whether Nutrient Synergy, a mixture containing ascorbic acid, lysine, proline, arginine, N-acetyl cysteine, and epigallocatechin gallate (from green tea extract), would enhance the cardio-protective effects of 17-? estradiol and progesterone.

Methodology:
The effects of hormones were investigated in cultured vascular smooth muscle cells (SMC) isolated from human aorta (Clonetics). SMC growth was stimulated by 10% fetal bovine serum and measured as DNA synthesis according to [3H] Thymidine incorporation into cellular DNA. SMC invasion assay was performed with metabolically pre-labeled cells penetrated through porous (8 micron pores) plastic membrane covered with natural extracellular matrix (Matrigel™, Becton Dickinson). Collagen synthesis was estimated by [3H] Proline incorporation into SMC culture media collagenase-sensitive protein. Cytokine secretion into SMC culture media was assayed with Quantikine ELISA kits (R&D Systems).

Results:
Estradiol and progesterone stimulated DNA synthesis in SMC (maximum increase of 30% and 24%, respectively) at concentrations 25-150 nmol/L. Cell growth stimulatory effects were attenuated with hormone concentrations increased up to 450 nmol/L. Nutrient Synergy at 20 mg/L (corresponding ascorbic acid content was 15 ?mol/L) inhibited SMC growth by 30% when used individually and reversed the stimulatory effect to inhibitory (25% maximum inhibition) when used with hormones. Dehydroepiandrosterone sulfate (DHEAS), a potential metabolic precursor of estradiol, inhibited SMC growth by 50% at 0.1 mmol/L. Addition of NS further enhanced the DHEAS inhibitory effect to 70% inhibition as compared to the control. DHEAS and P, but not E2, significantly increased SMC capacity to invade through Matrigel by 20% and 60%, respectively. Addition of NS reversed the stimulatory effects to inhibitory effects producing up to 60% inhibition of SMC invasion. In addition, NS reversed the effects of DHEAS on total collagen synthesis in SMC from 28% stimulation to 56% inhibition as compared to the control. E2 and P did not affect collagen synthesis when used individually. Moreover, E2, P and DHEAS slightly inhibited (up to 20% inhibition) tumor necrosis factor alpha-stimulated SMC secretion of such mediators of inflammation reaction as interleukin (IL) 1-beta, IL-6 and monocyte chemoattractant protein 1 in cultured media. The inhibitory effect was further enhanced by addition of NS.

Conclusion:
When tested in an experimental system of cultured human vascular smooth muscle cells, female sex hormones: estradiol, progesterone, and dehydroepiandrosterone sulfate, produced diverse effects on such atherogenic changes in SMC properties as pathologically increased growth rate and invasiveness, and excessive production of extracellular matrix components and inflammatory cytokines. Ascorbic acid-containing nutrient mixture, NS, enhanced the cardio-protective action of female sex hormones and counteracted their adverse effects at these experimental conditions. It remains to be tested whether this data could be confirmed in in vivo experimental settings.

Comment:
Large numbers of menopausal women are using hormone replacement therapy (HRT) for counteracting such adverse effects as hot flashes and loss of bone mass. However, HRT has been recently shown to have major adverse effects on cardiovascular health. When tested in an experimental system of cultured human vascular smooth muscle cells, female sex hormones: produced diverse effects on such atherogenic changes in smooth muscle cells properties as pathologically increased growth rate and invasiveness, and excessive production of extracellular matrix components and inflammatory cytokines. Nutrient Synergy enhanced cardio-protective action of female sex hormones and counteracted their adverse effects at these experimental conditions, suggesting it has great potential as an adjunctive therapeutic agent in inhibiting adverse effects and enhancing the cardio-protective effects of female sex hormones in HRT.