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α-Zearalanol, a Phytoestrogen for Cardiovascular Therapy

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Estrogen replacement therapy (ERT) is one of the most challenging issues women and their physicians have to face. Clinical and epidemiological studies have provided conflicting data regarding the cardiovascular benefit versus risk in women using ERT. Although ERT may improve several risk factors of coronary heart disease such as favorable changes in lipid profile, an associated increased incidence of uterine and breast tumors has jeopardized the clinical use of ERT. We reported here that the phytoestrogen α-zearalanol is effective against atherosclerotic development without overt growth-promoting effects in the uterus compared to estrogen. These results suggest clinical potential of this phytoestrogen as a "safe estrogen" with less risk of tumorogenesis.

Key Words: Phytoestrogen; estrogen; cardiovascular; atherosclerosis; uterine growth.

Introduction

Heart disease is the leading cause of death in women. Although cardiovascular mortality in men has declined, the number of cardiovascular deaths in women remains unchanged or even increasing. The onset of the clinical manifestations for cardiovascular diseases in women usually does not present for at least 10 yr after that of men. However, owing to an aging world population and a greater number of elderly women than elderly men, more women than men have suffered and ultimately died from cardiovascular diseases *(1)*. Many of the gender disparities in cardiovascular diseases have been attributed to the presence and function of the female sex hormones, especially estrogen. Over the past two decades, the impact of estrogen on the prevention and treatment of atherosclerosis, osteoporosis, Alzheimer's disease, and the aging has drawn some serious attention regarding its health benefits; however, the fact that estrogen replacement therapy (ERT) may predispose women to a much higher incidence of venous thrombosis and breast and endometrial cancers has undoubtedly compromised or jeopardized the clinical application of ERT. Although combined estrogen and progesterone therapy may reduce the incidence of endometrial cancer triggered by estrogen monotherapy, the estrogen-induced increase in the incidence of breast cancer remains elevated despite concurrent progesterone usage. While estrogen may benefit cardiac function, blood lipid profile, vascular resistance, blood pressure, and oxidative stress *(2)*, recent clinical trials such as the Heart and Estrogen/Progestin Replacement Study (HERS) have revealed unpredicted or even surprising findings regarding ERT on cardiovascular function *(3)*. While the pros and cons of ERT remain unclear and heavily debatable, the National Institutes of Health recently suspended the large clinical trials of ERT and the American Heart Association has publicly warned women of the risk of coronary heart disease with ERT *(1,4)*. Results of recent clinical trials demonstrated that ERT does not provide cardiovascular benefits in women with established heart disease. The general consensus is that women should not consider ERT without any known benefits of ERT for cardiovascular function. Thus, the search for safe and effective estrogen substitutes becomes a practical issue. Recently, the plant-derived phytoestrogens (which possess physiological properties of animal-derived estrogen) have been shown to act as potential replacements for estrogen. More than a hundred kinds of phytoestrogen have been identified since the 1950s, with genistein and isoflavone being most common. However, these phytoestrogens also have some negative effects on cardiovascular function, which cause potential clinical concern *(5)*.

Our published results *(6)* suggest that a novel phytoestrogen, α-zearalanol (α-ZAL), is a promising candidate for ERT. α-Zearalanol, a reductive product of the fungus *Gibberella zeae* metabolite zearalenone, was isolated from culture medium of zearalenone and belongs to the β-resorcylate family. α-ZAL may facilitate mouse uterine growth and promote weight gain in beef cattle and sheep *(7)*. Both α-ZAL and its parent compound zearalenone act as universal endogenous hormones for plant growth with α-ZAL being twice as effective as zearalenone, but less toxic *(8)*. α-ZAL promotes protein synthesis and increases the lean meat ratio

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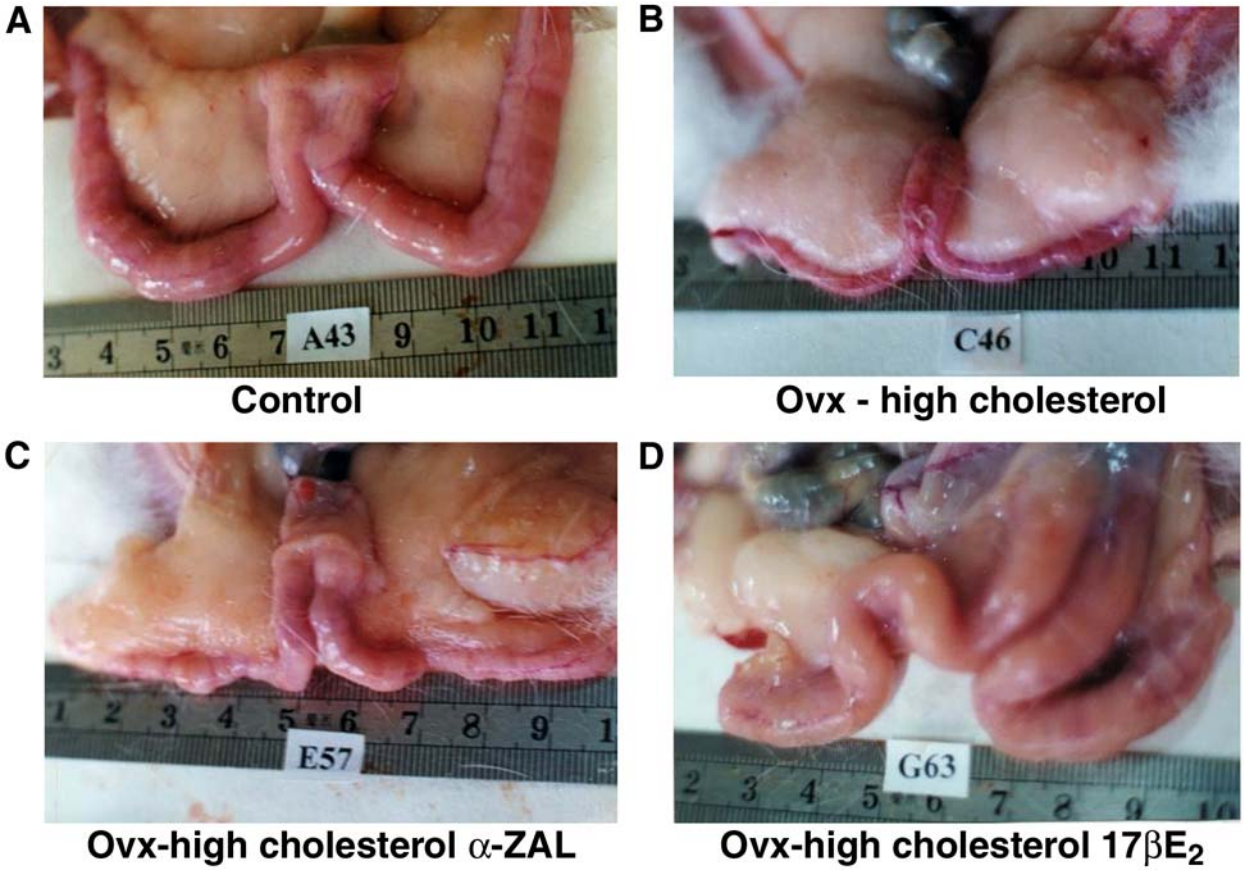

Fig. 1. Effect of α-ZAL and 17 β estradiol ($17\beta E_2$) feeding on uterine growth following bilateral ovariectomy (Ovx) and 12 wk of high cholesterol (2g/kg/d) dietary feeding. (**A**) Normal uterus; (**B**) atrophied uterus following ovariectomy and 12-wk cholesterol feeding; (**C**) ovariectomized uterus following 12-wk high cholesterol feeding supplemented with α-ZAL (0.5 mg/kg/d) (note that the size of uterus is greater than that in panel **B** but smaller than panel **A**) (**D**) ovariectomized uterus following 12-wk high cholesterol feeding supplemented with $17\beta E_2$ (0.5 mg/kg/d). Note that the size of the uterus is twice that of the normal uterus in panel **A** and 3.5 times the size of an α-ZAL-treated uterus in panel **C**.

of beef cattle and sheep in a manner similar to estrogen. However, little effect on tissue growth was noted for α-ZAL compared with estrogen. Taking advantage of their estrogen-like properties, both α-ZAL and zearalenone have been used as efficient and safe growth stimulants in diets in animal husbandry *(9)*. However, little is known of the medical value of these phytoestrogens from clinical studies.

In 1996, we postulated that α-ZAL be considered as an estrogen substitute because it possesses similar physiological properties of estrogen such as the prevention of atherosclerosis, osteoporosis, Alzheimer's disease, and delay of the aging process *(10,11)*. The real benefit of α-ZAL may be related to its relatively mild effects on the reproductive organs (e.g., breast and uterus). To further evaluate the clinical value of this phytoestrogen, we tested the effects of α-ZAL on atherosclerosis development in the ovariectomized, cholesterol-fed rabbit model *(6)*. Following 12 wk of high cholesterol feeding with or without α-ZAL (or estrogen) treatment (0.1–2.5 mg/kg/d), we found that cholesterol diet–induced atherosclerotic plaque formation was reduced by 50–85% (plaque area) by α-ZAL, which was as equally effective as estrogen. The plasma lipid total cholesterol, triglycerides, low-density lipoprotein-cholesterol (LDL-C), and Apo-protein B (ApoB) declined to various degrees compared to the high cholesterol diet group following α-ZAL treatment and this was associated with reduced aortic smooth muscle proliferation and extracellular Ca^{2+} invasion. These vascular protective effects of α-ZAL were comparable to or greater than those of estrogen *(6)*. Further mechanistic studies revealed that the α-ZAL-elicited protective effects may be related to inhibition of expression of c-myc mRNA and MCP-1 in smooth muscle cells, suppression of endothelin-1 (ET-1) release, modulation of endothelial nitric oxide (NO) production, and antagonism of oxidized LDL (ox-LDL)-induced downregulation of eNOS *(10)*. Perhaps the most intriguing and important finding from our study was that α-ZAL induced little enlargement of the uterus. The uterine enlargement elicited by α-ZAL was only approx 20% of that associated with equivalent doses of estrogen. The uterus (shown in Fig. 1) and mammary gland displayed little pathological change compared to treatment with 17β estrodiol. Unlike estrogen, α-ZAL had little effect on normal mouse mammary gland cell proliferation. In addition, α-ZAL significantly inhibited the expression of proliferat-

ing cell nuclear antigen (PCNA) and facilitated expression of tumor suppressing protein BINI mRNA (Y. Wu and W. Deng, unpublished data). There was no obvious adverse health effect or mortality during the duration of the α-ZAL treatment. These findings have both supported our initial hypothesis and broadened the research and developmental perspective of α-ZAL.

Mounting evidence indicates that zearalenone exists in many plants and vegetables including wheat, cotton, corn, celery, carrot and beet *(12)*. As an endogenous sex hormone, zearalenone is believed to play a significant role in herbal development and growth. It is worth mentioning that zearalenone was originally listed as a fungal mycotoxin and used as an index for seed and food contamination because the initial study found animals that consumed spoiled corn (containing zearalenone) displayed feminization and, in pigs, in which estrogen is the pregnancy-recognition signal, zearalenone induces pseudopregnancy *(13)*. Certain countries considered zearalenone as an exogenous substance for plant contamination and therefore restricted its agricultural applications. As an animal growth promoter, zearalenone has been studied with regards to its effects on organ and gland development *(14)*. Environmental hormones and their role in cardiovascular diseases have received special attention over the last 10 yr, especially with respect to preventing or reducing cardiovascular morbidity and mortality. As an environmental hormone, the endogenous zearalenone and its derivative α-ZAL have been the focus of this discussion. It has been reported that α-ZAL was able to reverse ovariectomy-induced endothelial dysfunction, alleviate DMBA-induced mammary gland tumor formation, and decrease the incidence of estrogen-dependent cancer *(14)*. As a non-steroidal estrogen, α-ZAL may lead to hemodynamic alterations, which may display toxicity in the kidney and/or liver. It appears that specific toxicities may be reduced or even reversed by rumen flora in digestive tract of ruminant species, but this is still under debate.

Our results suggested that α-ZAL may effectively protect blood vessels from lipid deposition in the tunica intima in a high lipid environment. A thorough system study on α-ZAL has not been performed. Our research in the past 5 yr has provided evidence for the use of α-ZAL in the prevention of atherosclerosis. To achieve a more rigorous evaluation of zearalenone and α-ZAL, future research should focus on the potential adverse effects of zearalenone and α-ZAL, and compared to those of estrogen. The results of these studies will benefit agriculture and animal husbandry, as well as human health.

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