Ph.D.-thesis by Claus Otto Lund, MD.

Title: Vascular effects of substances with estrogen-like action in a rabbit model.

Summary

The lower incidence of cardiovascular disease among women compared to men at the same age indicates a favourable influence of female sex hormones. Epidemiological studies of the associations between hormone replacement therapy (HRT) and cardiovascular disease suggest a considerable risk reduction of coronary vascular disease and a neutral effect on cerebrovascular disease. This is substantiated by biological studies in humans and animals showing beneficial alterations in lipoproteins, a direct anti-atherogenic effect and chronic and acute vasodilation after estrogen replacement therapy (ERT). However, randomised clinical trials published over the last five years demonstrate no preventive effect of HRT on ischemic heart disease or cerebrovascular disease.

The aim of the present study was to examine the effect of long-term treatment with estrogen or a dietary soy supplement (SoyLife®) on various aspects of vascular function and furthermore to develop a method for quantification of specific vascular mRNA transcripts. An additional aim was to evaluate the acute effects of tibolone (Livial®) and its three biologically active metabolites on vascular reactivity in cerebral and coronary arteries.

To mimic the situation in postmenopausal women, the study on long-term effects of hormone treatment was conducted in homozygous female Watanabe Heritable Hyperlipidemic Rabbits (WHHL), which develop endogenous hyperlipidemia and atherosclerosis on a standard diet due to a hereditary dysfunction of the LDL receptor. Ovariectomized WHHL rabbits were randomised to treatment with 17β-estradiol, SoyLife® or control given in a purified diet for 16 weeks. Posterior cerebral, basilar and proximal and distal coronary arteries were microdissected and total RNA was extracted and reversely transcribed. Ring segments of posterior cerebral and basilar arteries were mounted in myographs for isometric tension recordings. Plasma cholesterol was significantly higher at termination in the SoyLife® group, whereas LDL-cholesterol was comparable in all treatment groups. Plasma endothelin-1, measured at termination, was identical in all treatment groups. Using real-time PCR, endothelin receptor A and B mRNA expression showed no difference between treatment groups in the four different arteries. However, the results indicate that endothelin receptors were differentially expressed in proximal coronary arteries compared to the other arteries. Intriguingly, these differences coincide with the atherosclerotic lesions in the proximal coronary artery, suggesting an association between atherosclerosis and endothelin receptor expression. Myograph experiments indicated that either treatment influenced neither endothelium-dependent nor -independent relaxation in cerebral arteries. Correspondingly, eNOS mRNA was similarly expressed in all treatment groups. The findings are in opposition to some previous human and animal studies. Hypercholesterolemia may itself influence these parameters and may perhaps blunt the effects of the treatment. The findings may be unique to the WHHL rabbit in which the hypocholesterolemic effect of estrogens mediated by upregulation of liver LDL receptors is excluded.

In the second study, healthy adult female New Zealand White rabbits were sacrificed without prior intervention. Ring segment of cerebral and coronary artery were mounted in myographs for in vitro pharmacological experiments. Tibolone and its metabolites induced relaxation comparable to 17β-estradiol in both arteries with rapid onset, indicating non-genomic action. Investigations of possible mechanisms suggested that the relaxation was mediated by inhibition of voltage-gated calcium channels and possibly partly by nitric oxide release. However, the concentrations of tibolone

producing vasodilation in vitro were several magnitudes higher than the circulating plasma concentrations of tibolone achieved in the clinical setting. The current results may therefore reflect a pharmacological rather than a physiological effect of tibolone.