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Selective Estrogen Receptor Modulators and Coronary Heart Disease

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The vasculature has been recognized as an important target of estrogen action through rapid non-genomic effects and/or via the classic pathway (genomic effects) involving estrogen receptors (ER-α and ER-β). Multiple mechanisms participate in the regulation of different estrogen-controlled genes, providing a wide spectrum of possibilities for development of drugs, including pure agonists or antagonists or mixed agonists/antagonists, the so-called selective estrogen receptor modulators (SERM). In theory, an ideal SERM should reduce the risks of coronary heart disease (CHD) and preserve bone density, without or with very low incidences of breast and endometrial neoplasms or venous thromboembolism (VTE). The precise mechanism for the protective effects of estrogens and their receptors on cardiovascular diseases is not yet fully established. In this review, we summarize the recent advances in understanding the action of ERs/ligands, the therapeutic implications for CHD, and highlight the recent progress of both clinical and basic studies on the protection issue. Finally, a number of newly developed SERMs and their clinical applications as well as the laboratory investigations are discussed. (Trends Cardiovasc Med 2001;11:196–202). *© 2001, Elsevier Science Inc.*

The vasculature, like the reproductive tissue, bone, liver, and brain, has been recognized as an important target of estrogen action through rapid non-genomic effects and/or via the classic pathway (genomic effects) involving estrogen receptors (ER-α and ER-β). Multiple mechanisms participate in the regulation of different estrogen-controlled genes, providing a wide spectrum of possibilities for development of drugs, including pure agonists or antagonists or mixed agonists/antagonists, the so-called selective estrogen receptor modulators (SERM). Coronary heart disease (CHD), representing the majority of cardiovascular diseases (CVD), is the leading cause of death in both women and men. It has been widely accepted that estrogen can reduce the risk of CVD in women, a notion that was supported by a large body of epidemiological studies. However, this concept has been unexpectedly challenged, because apart from some positive effects on lipid profile, there was no significant improvement of CVD in postmenopausal women receiving hormone replacement therapy (HRT), as reported recently from the first large randomized clinical trial,

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the HERS study (Hulley et al. 1998). Thus, development of new drugs (e.g., SERMs) becomes an extremely important issue for the pharmaceutical industry. This review summarizes recent advances in understanding the action of ERs/ligands, the therapeutic implications for CHD and the development of SERMs.

• Molecular Basis of Estrogen Receptor Modulators

Since the second estrogen receptor (ER-β) was discovered in 1995 (Kuiper et al. 1996), worldwide research efforts have increased dramatically, aiming at reevaluating and understanding the physiological/pathophysiological role of estrogen, mediated by both ERs (ER-α and ER-β). Human ER-β shows approximately 89% identity with rat ER-β, 88% identity with mouse ER-β, and 47% identity with human ER-α. ER-α shows highest expression in uterus, testis, pituitary, ovary, epididymis and adrenal, whereas ER-β is highly expressed in brain, kidney, prostate, ovary, lung, bladder, intestine and epididymis (Enmark and Gustafsson 1999). Estrogen receptor was originally viewed primarily as a transcription factor, simply switching expression of target genes on or off (Tsai and O'Malley 1994). However, recent knowledge suggests that the biological action of ERs/ligands is far more complicated than we previously thought. The novel mechanisms of ER/ligand-action have been reviewed recently (MacGregor and Jordan 1998). At present, estrogen is no longer viewed just as a female sex hormone but rather as a steroid hormone functioning in both females and males.

A detailed description of recently acquired knowledge regarding the mechanism of estrogen action is, however, beyond the scope of this review. In summary, the interactions between ERs and their ligands have the following important characteristics: (1) the isoform-specific tissue distribution of ERs (Couse et al. 1997); (2) the multiple isoforms of both ERs (Chu and Fuller 1997, Lu et al. 1999); (3) the capacity of ERs to form heterodimers and homodimers (Pettersson et al. 1997); (4) the ligand-binding-dependent conformational changes of ERs (Beekman et al. 1993); (5) the distinct binding affinities of ERs to different ligands (Kuiper et al. 1998); and (6) the involvement of tissue-specific co-activators and co-repressors (Chen and Li 1998). The efficiency of estrogen in modulating gene transcription is dependent on the interactions between ERs and co-regulator proteins.

The co-repressors bind to ERs when they are liganded with an estrogen antagonist (Montano et al. 1999). Estrogen agonists induce a conformation in ERs that stabilizes co-activator binding. A group of co-activator peptides has been demonstrated in vitro to markedly stabilize the binding of ER and its agonist ligands but not ER-antagonist complexes (Gee et al. 1999). In addition, it has recently been reported that the transcriptional activation by human ER-α and ER-β is cell type- and promoter-dependent (Jones et al. 1999). Structural analysis of ER's ligand-binding domain (LBD), in the presence of 17β-estradiol, raloxifene, or genistein, provided insight into the mechanisms of agonism and antagonism in ERs (Shiau et al. 1998). 17β-estradiol can function as an agonist regardless of whether AF-1 or AF-2 (activation function -1 or -2) is the dominant activator. Tamoxifen inhibits AF-2 activity and consequently functions as an antagonist in all situations where AF-2 is required. On the other hand, tamoxifen manifests partial agonist activity if AF-1 is the dominant activator. The pure antiestrogen ICI 182,780 inhibits the activity of both AF-1 and AF-2 and almost completely blocks the ability of ER-α to activate transcription through classic ERE-mediated pathways (Barkhem et al. 1998). ERs do not just change from an inactive to an active form upon binding a ligand, but are quite malleable and can exist in several different ligand-induced conformations. This complexity has made it possible to develop new drugs (e.g., SERMs), which act as estrogens in some tissues but as antiestrogens in others.

In addition to the classic ligand-activated pathway, it has been shown that ER can be activated by growth factors in the absence of ligand (Aronica and Katzenellenbogen 1993). Apart from signaling through ERs, when estrogen effects are seen hours after hormone administration, the rapid non-genomic estrogen effects were first reported in 1977 with the finding of a cell membrane-localized estrogen receptor (Pietras and Szego 1977). It has become recognized that estrogen can rapidly induce elevated levels of intracellular secondary messengers (e.g., calcium and cAMP), as well as activate mitogen-activated protein (MAP) kinase and phospholipase. The rapid vasodilating effect of estrogen is at least partially related to its ability to enhance the bio-availability of nitric oxide (NO) (Guetta et al. 1997) and to open calcium-activated potassium channels (White et al. 1995). These issues have been reviewed recently (Collins and Webb 1999).

• Pathophysiological Effects of Estrogen on CVD

It has been well established that premenopausal women have a lower risk to develop CHD than age-matched men (Corrao et al. 1990). Differences between men and women with regard to CHD tend to diminish after women reach menopause (Samaan and Crawford 1995). Functional ERs were found in vascular smooth muscle (VSM) cells, myocytes as well as fibroblasts (Grohe et al. 1997, Karas et al. 1994). In cardiac myocytes expression of ER-α is influenced by gender, whereas ER-β shows no sexually dimorphic expression pattern (Grohe et al. 1998). Estrogen is documented to play a critical role in atheroprotection (Stampfer et al. 1991), owing to its direct and/or indirect effects on vascular integrity. The mechanisms include decrease of vascular tone, activation or inactivation of NO/NOS pathway; cytoprotective effects on the cells of the arterial wall, inhibition of migration of myocytes and proliferation of fibroblasts; elevation of HDL cholesterol, decrease of LDL cholesterol and lipoprotein, and inhibition of LDL oxidation; decrease of plasminogen and fibrinogen concentrations (Mendelsohn and Karas 1999).

An additional possible explanation for the apparent protection by estrogens of premenopausal women against the development of coronary atherosclerosis is the "iron hypothesis" (Sempos et al. 1994). Iron is a powerful oxidant that modulates lipid-peroxidation reactions to increase formation of oxidized LDL cholesterol, which in turn is implicated in the initiation and progression of atherosclerosis. It is well established that estrogens have a major antioxidant effect on lipid substrates, owing to the aromatic hydroxyphenol structure of the A-ring of the steroid molecule (Negre-Salvayre et al. 1993). This "oxidant mechanism" was supported by the data from a clinical study, in which 38 postmenopausal women received estrogen therapy for 6 months. A significant increase of thiobarbituric acid-reactive substance concentration and glutathione peroxidase activity, together with a significant decrease in the activity of glutathione reductase and superoxide dismutase was observed (Akcay et al. 2000). Recently, two new mechanisms have been suggested for the anti-atherogenic action of estrogen, namely (1) a decrease in endothelial layer permeability, and (2) formation of estrogen/LDL complexes and prevention of LDL binding to the arterial wall (Walsh et al. 2000).

In animal studies, it has been shown that estrogen increases the growth of endothelial cells after denudation (Krasinski et al. 1997), reduces neointimal proliferation (Foegh et al. 1994), and reduces the size of vascular lesions in carotid arteries in mice (Sullivan et al. 1995). The endothelial NOS (eNOS) and inducible NOS (iNOS) have been identified as estrogenic downstream target gene products in myocardium (Weiner et al. 1994). It has been suggested that the short-term effects of estrogen on the cardiovascular system are mediated by ER-α in a non-genomic manner, by activating eNOS through a MAP kinase-dependent mechanism (Chen et al. 1999). This notion was supported by clinical findings: a male patient with mutated ER-α has impaired endothelial function and suffers from pre-atherosclerosis (Sudhir et al. 1997). Furthermore, estrogen has been shown to modulate smooth muscle cell responses to vascular injury in experimental mice lacking ER-α (Iafrati et al. 1997), suggesting that ER-β, rather than ER-α, mediates the response to estrogen in this context. This was further confirmed in another mouse study, which showed an increased expression of ER-β mRNA in blood vessels after vascular injury (Lindner et al. 1998). More recently, when the same vascular injury model was used in ER-β knockout mice, it was observed that ER-β is not either required for estrogen-mediated inhibition of responses to vascular injury, suggesting that ER-β and ER-α may substitute for each other, respectively, or that a third ER (ER-γ) may exist, or that estrogen operates via an ER-independent mechanism (Karas et al. 1999). Further mechanistic insight into the vasculoprotective effects of estrogen may be achieved through similar experiments with ER-α/ER-β double-KO animals.

• Therapeutic Implications

Hypertension is one of the established risk factors of CHD, which is less common and severe in premenopausal women than in men of similar age (Corrao et al. 1990). The blood pressure in postmenopausal women gradually catches up with that in men, and is even higher by the sixth decade of life, suggesting that endogenous estrogen and/or progesterone may play a role in prevention of hypertension (August and Oparil 1999). It was recently reported that HRT was effective in reducing blood pressure in 34 postmenopausal women with treated hypertension, possibly owing to blocking of calcium channels (Szekacs et al. 2000). HRT also decreased the blood pressure in postmenopausal women with mild to moderate hypertension (Modena et al. 2000), perhaps owing to the increased circulating plasma levels of bradykinin (Sumino et al. 1999). In the arterial endothelium, estrogen increases eNOS activity and production of NO, which has favorable effects on arterial vasomotor tonus (Caulin-Glaser et al. 1997).

Both ERs, especially the newly discovered estrogen receptor ER-β, have been shown to be distributed widely in the body in both genders (as reviewed above). It seems that ER-α is more prevalent than ER-β in female human VSM cells, whereas the expression of the two ER subtypes is equal in male VSM cells (Grohe et al. 1998). Both ERs in cardiac myocytes from male and female rats were upregulated by estrogen treatment (Grohe et al. 1997). The cardiovascular actions of estrogen in males are well recognized (Sudhir and Komesaroff 1999). Acute estrogen treatment has been shown to abolish the abnormal cold-induced coronary vasoconstriction and improve coronary blood flow in men (Reis et al. 1998) and increase acetylcholine-induced but not metabolic vasodilatation in males (New et al. 1999). These favorable vasomotor effects may result from direct estrogen action on vasculature tone (Guetta et al. 1997). Studies on estrogen-treated biological males provide a unique opportunity to investigate the long-term action of estrogen in males. No increased risk of cardiovascular morbidity or mortality was seen in a cohort of over 800 male-to-female transsexuals prescribed estrogen (van Kesteren et al. 1997). A marked increase of ER-α mRNA was reported in the male New Zealand rabbit cardiac-aorta allografts from male Dutch Belted rabbits, suggesting that estrogen may have protective effects mediated by ER-α (Lou et al. 1998).

HRT administered to postmenopausal women relieves climacteric symptoms, prevents loss of bone mass, and counteracts the development of coronary artery disease. Although HRT has proven beneficial in reducing a number of the known risks of CHD, the associated side effects (e.g., venous thromboembolism [VTE]; Hoibraaten et al. 1999) and no net benefit as claimed by the HERS study (Hulley et al. 1998), have raised a lot of questions regarding currently available drugs. Additional drug trials of sufficient size and length of treatment are needed to further clarify the HRT issue. At present, postmenopausal women with developed CHD should not randomly obtain hormone therapy until the additional large trials of HRT are completed in the next several years. The statistical analysis, interpretations as well as limitations of the HERS study have been addressed lately (Foody 1999, Kooistra and Emeis 1999, Seed 1999).

• SERMs and CHD—Perspectives

A recent meta-analysis of the effects of soy consumption on lipid levels confirmed the general trends of this type of study, investigating the alleged positive effects of soy proteins on cholesterol levels (Anderson et al. 1995). However, the lipid-lowering effect of phytoestrogens may not be their only mechanism of cardiac protection. Recently it has been reported that soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques similarly to estrogen (Honore et al. 1997). The dilatation response to acetylcholine is absent in ovariectomized (VOX) and male monkeys. Therefore, the effect of soy isoflavones in enhancing vascular reactivity may depend on the presence of some circulating estrogen. The two primary soy phytoestrogens are genistein and daidzein, both of which bind to estrogen receptors with low to moderate affinity. The isoflavone genistein has been tested in vitro for binding to both ERs, and the affinity for ER-β was 20-fold higher than for ER-α (Makela et al. 1999). In addition, genistein was shown to cause a selective protection against vascular injury with no uterotrophic effect in mice when compared to 17β-estradiol. A striking ER subtype-selective activity of genistein is its partial agonism, mediated by ER-β, but slight superagonistic activity when bound to ER-α (Barkhem et al. 1998). The plant-derived estrogens may exert both estrogenic and anti-estrogenic effects in humans, depending on several factors, including their concentration, the concentrations of endogenous estrogens, and individual characteristics, such as gender and menopausal status (Knight and Eden 1996). As it seems, soy consumption offers a safe, inexpensive and generally side effect-free alternative to current pharmaceutical therapy.

At present, the designed "ideal estrogens" or SERMs are subject to much attention from large pharmaceutical industries. The SERMs target desired tissues, such as the cardiovascular system and bone, acting as estrogen agonists in these organs, while they act as antiestrogens in uterus and breast, with very few side effects or none. The potential benefits of these drugs include protection against four important hormone-dependent diseases: CHD, osteoporosis, and endometrial as well as breast cancer, which have a strong impact on women in terms of morbidity and mortality.

Raloxifene (a nonsteroidal benzothiophene derivative), binds to and interacts with estrogen receptors, acting as an estrogen agonist in bone and liver, but as an estrogen antagonist in breast and uterus (Bryant and Dere 1998) (see Figure 1). Therefore, raloxifene as a key member of SERMs represents a potentially important alternative to conventional HRT in postmenopausal women for the prevention and treatment of osteoporosis and cardiovascular disease (Gustafsson 1998). It has been shown that raloxifene acutely relaxes rabbit coronary arteries in vitro in an estrogen- and NO-dependent manner, reduces cholesterol content in arteries (Figtree et al. 1999), and inhibits LDL oxidation in rabbits (Bjarnason et al. 1997). Published data from two independent clinical trials with raloxifene have shown that raloxifene can decrease plasma LDL cholesterol and fibrinogen (de Valk-de Roo et al. 1999), and also decrease homocysteine without changing the plasma levels of C-reactive protein in postmenopausal women (Walsh et al. 2000). However, it failed to show a reduced size of coronary artery plaques in VOX monkeys after a 2-year raloxifene treatment (1 mg or 5 mg/kg per day) as compared to the estrogen-treated group (Clarkson et al. 1998). In summary, raloxifene can favorably alter biochemical markers of cardiovascular risk. However, its protective action against cardiovascular disease requires further investigation. To address the ability of raloxifene (Evista) to prevent heart attacks and heart-related deaths in postmenopausal women the RUTH (*r*aloxifene *u*se for *t*he *h*eart) study was initiated in 1998. This study will enroll approximately 10,000 postmenopausal women at risk of heart disease and last for more than 7 years.

Several new SERMs for prevention and/or treatment of serious postmenopausal health risks are in development. EM800 (SCH57050) and EM-652 (SCH

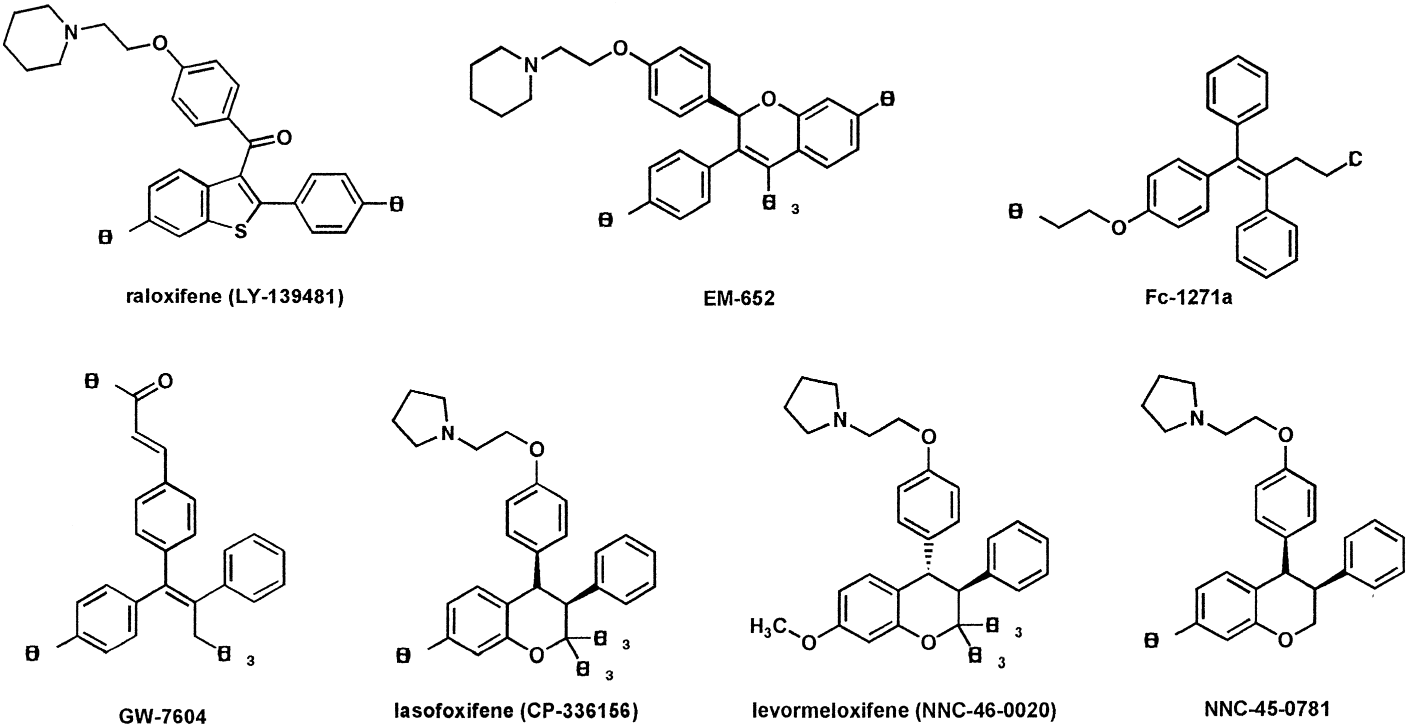

Figure 1. Structure of some of the SERMSs mentioned in the text. Raloxifene is the only SERM that today is used in the clinic for prevention and treatment of postmenopausal osteoporosis. All the other SERMs depicted are either in clinical development or have been withdrawn (Levormeloxifene) from further development due to severe or unacceptable side effects.

57068) preserve several estrogenic functions (Labrie et al. 1999). These nonsteroidal compounds act as pure estrogen antagonists to both ER-α and -β on gene transactivation via an estrogen response element, with similar potency for both ER subtypes (Tremblay et al. 1998). GW5638 and GW7604 are two other SERMs, claimed to have estrogen agonist activity in bone and the cardiovascular system (Willson et al. 1997). CP-336156 (Lasofoxifene) (Rosati et al. 1998), a nonsteroidal estrogen with mixed agonist/antagonist activity, is in clinical development for prevention of osteoporosis and for treatment of breast cancer. CP-336156 has also been shown to be as potent and efficacious as 17β-estradiol to lower total serum cholesterol in rats. The novel triphenylethylene compound FC1271a has a tissue-selective estrogen agonist profile (Qu et al. 2000). A novel chroman analogue, NNC 45-0781, was demonstrated to have full anti-resorptive effect at distal femora in VOX rats and was shown to partially reverse established osteopenia in aged rats (Wassermann et al. 1998). NNC 45-0781 was also reported to have no estrogenic effect on breast or uterine tissue but had, like estrogen, a lowering effect on the serum cholesterol levels. Perhaps unexpectedly for a non-steroidal estrogen with tissue-selective, mixed agonist/antagonist activity, NNC 45-0781 attenuated a rise in the tail skin temperature in the morphine-addicted rat model for postmenopausal hot flashes.

Additional SERMs are in clinical development (Bryant and Dere 1998), but a serious drawback of this kind of drug became evident when the SERM levormeloxifene had to be withdrawn from further clinical development owing to increased incidence of urinary incontinence and uterine prolapse in postmenopausal women (note in SCRIP No 2374, 1998, page 18). The mechanistic explanation for the adverse gynecological events with levormeloxifene is unknown. However, future attempts to develop the "ideal" estrogen or SERM will have to take into account that there are two estrogen receptors, ER-α and ER-β, involved in mediating the biological effects of estrogens and antiestrogens. ER subtype-selective SERMs will probably better provide the benefits of estrogen replacement therapy with reduced side effects. Several of the large pharmaceutical companies are engaged in the development of ER-α- and ER-β-selective SERMs (third generation HRT), but as yet there is no information as to how far they have come in their development. However, synthetic ER subtype-specific ligands have been reported (Sun et al. 1999). The most ER-α-selective ligand showed 120-fold higher agonist potency for ER-α than for ER-β. Another, ER subtype-selective ligand synthesized by the same group showed full ER-α agonism but pure ER-β antagonism (Meyers et al. 1999).

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Matrix Metalloproteinase Inhibition and the Prevention of Heart Failure

Richard T. Lee*

Matrix metalloproteinases (MMPs) are members of a large family of enzymes that can degrade extracellular matrix as well as other molecules. MMPs participate in a broad variety of normal and pathologic states, and recent evidence implicates the MMP family as potential mediators of cardiac dilation and progression to heart failure. This evidence is based on several lines of investigation. First, members of the MMP family are overexpressed in the myocardium in both experimental and human myocardial injury, infarction, and dilation. Second, overexpression of at least one MMP (MMP-1) in the hearts of transgenic mice can cause cardiac hypertrophy, dilation, and systolic dysfunction. Third, studies from multiple laboratories with different experimental models indicate that inhibition of MMPs through small molecules or gene transfer of endogenous inhibitors favorably affects cardiac remodeling. Fourth, targeted deletion of MMP genes in mice attenuates cardiac remodeling. These compelling results appear to fulfill Koch's Postulates as they may be applied to a non-infectious mediator of a disease, and thus current evidence supports MMP inhibition as a promising strategy for preventing heart failure. However, the crucial question of whether MMP inhibition benefits long-term left ventricular function and survival should be answered. (Trends Cardiovasc Med 2001;11:202–205). © *2001, Elsevier Science Inc.*

The realization that the extracellular matrix is a dynamic environment in normal conditions—and particularly active in response to injurious stimuli—has led to the emergence of therapeutic strategies of modifying extracellular matrix in a broad variety of diseases. The extracellular matrix serves not only as the substrate for cell adhesion and the foundation for tissue structure, but also as a critical cell signaling environment. Advances in the biology of matrix metalloproteinases (MMPs) as well as development of small molecule MMP inhibitors have led to clinical development of these compounds. Although clinical studies of MMP inhibition have thus far been primarily limited to connective tissues diseases and cancer, the evidence that MMP inhibition can prevent congestive heart failure is growing (Mann and Spinale 1998).

MMPs are members of a family of zinc-dependent enzymes that degrade specific components of the extracellular matrix (Nelson et al. 2000). At least 20 different MMPs have been identified, and the majority of these enzymes are secreted as inactive zymogens that require

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