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27-Hydroxycholesterol: the First Identified Endogenous SERM

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Abstract

The cholesterol metabolite 27-hydroxycholesterol (27HC) classically delivers sterols from peripheral tissues to the liver and is a substrate for bile acid synthesis. Recent studies have revealed that 27HC also uniquely binds to and modifies estrogen receptor (ER) α and ER β function. Experiments in mice lacking Cyp27a1, which synthesizes 27HC, or Cyp7b1 that catabolizes 27HC have demonstrated that 27HC adversely impacts estrogen-related cardiovascular protection and bone mineralization. Work in breast cancer cells further indicates that 27HC alters ER target gene expression to promote cell growth. Therefore, 27HC is the first identified endogenous selective estrogen receptor modulator (SERM), and has potentially important impact on the cardiovascular system, bone biology, and cancer.

Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators, or SERMs, have both agonist and antagonist activities involving high affinity interactions with estrogen receptors (ER), and they cause unique changes in ER conformation that are distinct from those induced by receptor binding by naturally-occurring forms of estrogen. There is resulting selective recruitment and interaction of either ER α or ER β with coactivators and corepressors, and SERMs thereby cause varied responses that are cell-type selective and gene target-specific, and both agonistic and antagonistic in nature [1]. The biological actions of the first SERM clomifene were described in the early 1960s. More than 20 years later in 1986 when human ER α was cloned, it became possible to understand the transcriptional activity of the receptor, and therefore also the actions of synthetic SERMs such as tamoxifen and raloxifene [1,2]. Another twenty years later in 2007 it was discovered that there is a naturally-occurring SERM, the oxysterol 27-hydroxycholesterol (27HC), which is an abundant metabolite of cholesterol [3]. The recent study of 27HC as a SERM, both in cell culture and in genetically-modified mice, has provided new understanding of an additional dimension in the complex nature of ER action in health and disease.

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27HC as an Oxysterol

Oxysterols are metabolites of cholesterol containing a hydroxyl group on the side chain that serve multiple roles in lipid metabolism. They function in reverse cholesterol transport to deliver sterols from peripheral tissues to the liver, primarily as components of low density lipoprotein cholesterol (LDL) or high density lipoprotein cholesterol (HDL) particles and mostly in esterified form due to the actions of lecithin-cholesterol acyltransferase (LCAT). In addition, they are key substrates for bile acids synthesis, and therefore have important extrahepatic as well as intrahepatic roles in the elimination of excess cholesterol. Oxysterols may also alter cholesterol synthesis, with evidence that they bind to Insig (insulin-induced gene) and thereby block sterol regulatory element-binding protein (SREBP)-mediated mechanisms for the regulation of sterol-sensitive genes, and that they increase the degradation of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase [4,5]. Furthermore, oxysterols may function as ligands for the liver X receptors (LXR) α and β , and thereby have additional potential impact on cholesterol homeostasis [6]. However, whether such negative feedback mechanisms play a significant role in cholesterol homeostasis in vivo is unclear because genetically-modified mice with markedly reduced or elevated oxysterol levels do not have significant alterations in global cholesterol homeostasis [7,8]. The most abundant oxysterols are 24-hydroxycholesterol (24HC), 25-hydroxycholesterol (25HC), and 27HC, with 27HC being the most prevalent [4,9,10].

27HC is generated from cholesterol enzymatically by the P450 enzyme sterol 27-hydroxylase encoded by Cyp27a1, which is abundant in the liver where it contributes 27HC as substrate for bile acid synthesis. In addition, Cyp27a1 is expressed in the intestine, vasculature, brain, and macrophages [9], indicating local regulation of 27HC levels in nonhepatic tissues and cell types. Individuals with mutations in Cyp27a1 accumulate cholesterol and cholestanol in the brain and tendons in a condition known as cerebrotendinous xanthomatosis. This occurs because the resulting reduction in bile acid synthesis due to defective Cyp27a1 causes a compensatory increase in the activity of cholesterol 7 α -hydroxylase [11].

Regarding oxysterol catabolism, 27HC and the other prevalent oxysterol 25HC are both hydroxylated by oxysterol 7 α -hydroxylase encoded by Cyp7b1 which is abundant in the liver, and the resulting 7 α -hydroxylated forms are ultimately metabolized to bile acids [4,8,9]. Patients with mutations in the Cyp7b1 gene and impaired hydroxylation of 27HC have circulating levels of 27HC of 2–3 μ M compared with normal concentrations of 0.072–0.73 μ M [3,12–14]. Mice that are null for Cyp7b1 have 3- to 5-fold elevated levels of 27HC and 25HC. Despite having attenuated 27HC and 25HC hydroxylation, Cyp7b1^{-/-} mice have normal plasma cholesterol and triglyceride levels, and this is likely due to increases in bile acid synthesis via other pathways [8]. In addition to its primary role in the metabolism of oxysterols in the liver, Cyp7b1 is expressed in other tissues including the kidney and lung where it may locally catalyze the 7 α -hydroxylation of 27HC, 25HC, or other substrates [15]. The latter potentially include pregnenolone and dehydroepiandrosterone (DHEA), which are neurosteroid and sex hormone precursors. An additional putative substrate for Cyp7b1 is 5 α -androstane-3 β ,17 β -diol, or 3 β Adiol, which is an ER ligand in prostate cells whose metabolism by Cyp7b1 terminates its estrogenic activity. However, the evidence that there are other substrates for Cyp7b1 besides 25HC and 27HC arises solely from ex vivo and in vitro experiments, and these processes have not been demonstrated in vivo [16]. It is important to note that there are two lines of Cyp7b1^{-/-} mice. One line displays a hyperestrogenic phenotype in mammary gland and uterus [17], whereas the other does not [8]. The basis for these differences remains to be determined.

27HC as a SERM

Up until recently, the only recognized SERMs were synthetic molecules. The first evidence that 27HC is a SERM came from Gal4-ER cotransfection assays testing ER α and ER β function in HEK293 cells. Of the series of abundant endogenous oxysterols tested, 22R-hydroxycholesterol, 24S-hydroxycholesterol, 25HC and 27HC inhibited 17 β -estradiol (E₂) activation of ER α and ER β with IC₅₀ values ranging from 1–5 μ M for both ERs. 27HC was the most potent oxysterol (IC₅₀ = 1 μ M), with 90% efficacy on ER β and 50% efficacy on ER α . Importantly, 27HC did not display inhibitory effects with any other human nuclear receptors. [³H]-E₂ competition ligand-binding assays revealed that 27HC binds directly to purified recombinant ER α (K_i= 1.32 μ M) and ER β (K_i=0.42 μ M), and competition with ICI182,780 but not with the oxysterol 7-keto-cholesterol demonstrated specificity of 27HC binding to ER. Mammalian 2-hybrid assays further showed that 27HC inhibits E₂-induced interaction of the coactivator SRC-1 with ER β but not with ER α [3]. In addition, in HepG2 cells cotransfected with ER α fused to the VP16 transactivation domain (VP16-ER α), with coactivator peptides fused to the Gal4 DNA-binding domain (Gal4DBD) and with a 5XGal4Luc3 luciferase reporter, treatment with 27HC led to the recruitment of a number of coactivators to the receptor [18]. Furthermore, it was demonstrated that the impact of 27HC on ER α and ER β transactivation via classical estrogen response elements (ERE) varies in diverse cell types. Whereas in the presence of E₂ 27HC displays partial antagonist action on ERE-mediated transcriptional transactivation in bovine aortic endothelial cells (BAEC) and MCF-7 human breast cancer cells, 27HC behaves as an agonist in HepG2, Caco-2, and HeLa cells [3,18]. However, in the absence of E₂ in ER α -expressing HeLa cells, 27HC stimulates ERE-mediated transactivation, but with potency far below that of E₂. As such, 27HC has mixed function, and the cellular context and target of action are important variables in understanding SERM activity.

To provide further biochemical proof of 27HC behavior as a SERM, ligand-induced conformational changes in ER were investigated using peptide probes that bind to differentially-exposed protein-protein interaction surfaces. In mammalian 2-hybrid assays, certain peptides known to associate with ER α upon its interaction with E₂ or synthetic ligands also displayed association with the receptor when it was bound to 27HC, whereas other peptides did not. As importantly, combinatorial peptide phage display identified peptide probes that preferentially bound to ER α in the presence of 27HC versus E₂ or a synthetic SERM [18]. These collective observations revealed that 27HC induces a unique active conformation of ER α , thereby providing additional evidence that 27HC is a novel SERM.

27HC Levels in Health and Disease

As noted, the majority of circulating 27HC is transported in association with LDL and HDL cholesterol. There are no significant diurnal changes, and in healthy volunteers, plasma and the non-HDL subfraction concentrations of 27HC are strongly correlated with the concentrations of cholesterol, non-HDL cholesterol and triglycerides. Circulating levels of 27HC increase with age particularly after the age of 30, and they are believed to be derived primarily from extrahepatic sources [10,19,20]. Compared with plasma levels of 27HC, their abundance in atherosclerotic lesions has been found to be orders of magnitude greater, approaching millimolar concentrations in developing foam cells and atherosclerotic plaques [10]. Along with 27HC, its synthetic enzyme Cyp27a1 is abundant in atherosclerotic lesions, in both endothelium and macrophages [21]. The amount of 27HC found in lesions increases with the severity of the lesion and with the abundance of macrophages [21,22]. Although the majority of 27HC in plasma and in atherosclerotic plaques is esterified, a significant portion is free [8,10].

Circulating 27HC levels are lower in females than in males in both rodents and man, and this most likely relates to the upregulation of Cyp7b1 expression by E₂ and ER [13,23]. Mirroring the correlations between circulating cholesterol levels and 27HC observed in humans, studies in mice reveal how 27HC content in tissues is impacted by lipid status. The provision of a high cholesterol/high fat (HC/HF) diet (21% milk fat, 0.2% cholesterol) to wild-type mice for 8 weeks causes a 3-fold increase in aorta total 27HC content, with the percent of total 27HC that is unesterified being 41% on chow diet and 33% on HC/HF diet. Estimating aortic tissue density to be 0.8–1.0g/ml, even in the absence of atherosclerotic lesion development hypercholesterolemia causes total and unesterified 27HC in the vasculature to increase to 1.9 and 0.6 uM, respectively, which approach the IC₅₀ for actions on ER [3]. As such, vascular 27HC content increases with hypercholesterolemia to concentrations that would be expected to modify ER function.

Impact of 27HC on the Cardiovascular System

ER regulate many physiologic processes besides reproduction [24], and the cardiovascular system is a potentially important physiologic as well as pharmacologic target of ER action, where the effects of E₂ on endothelial and vascular smooth muscle (VSM) cells are believed to be beneficial [25]. In mice ER α is required for the maintenance and repair of vascular endothelium, through actions that include the upregulation of endothelial NO synthase (eNOS) expression and the unique stimulation of the enzymatic activity of existing eNOS caused by non-nuclear ER signaling that activates kinase signaling in endothelial cells [26–28]. ER β mediates estrogen-dependent VSM dilation via the upregulation of VSM inducible NOS (iNOS) and effects on VSM potassium channels [29]. Experimental evidence from animal studies [30] and in both men and women [31,32] indicate a cardioprotective role for estrogen. Nevertheless, estrogen use in postmenopausal hormone replacement therapy (HRT) remains controversial [33,34], mainly owing to the results of two large clinical trials of HRT that did not show a benefit but rather an increased risk of cardiovascular disease [35,36]. A key variable that may explain the differences between the observational and randomized clinical trials is the age at which women initiated treatment [31]. Most women in the observational studies were in the perimenopausal or early postmenopausal period at the time of initiating HRT, whereas subjects in the large trials were 12 to 18 years postmenopausal [33]. The “timing hypothesis” addresses this potentially important difference in cardiovascular outcome between the observational and randomized clinical trials, stating that HRT is not beneficial when given to older women [31], and later analysis of the randomized studies revealed favorable effects of HRT when initiated within 10 years postmenopause [37]. An additional potentially important and related variable is the likely presence of preclinical atherosclerosis in the subjects in the large clinical trials. Whereas U.S. women have only fatty streaks and minimal atherosclerotic plaques in their coronary arteries at age 35, there is progression of lesion formation between ages 45 and 55, and more complex lesions are present by age 65 [38–40]. The adverse impact of preexisting atherosclerosis on estrogen-related cardiovascular protection has been well-documented in nonhuman primate studies [41].

The lack of efficacy of HRT in the trials that were likely confounded by preclinical vascular disease, the animal studies showing attenuated estrogen action upon existing atherosclerosis, the recognition that 27HC accumulates markedly in atherosclerotic lesions, and the biochemical evidence that 27HC is a SERM all led to the hypothesis that 27HC antagonizes the favorable cardiovascular actions of estrogen. This possibility was interrogated using numerous approaches focused on E₂ modulation of vascular eNOS and iNOS. It was demonstrated that whereas eNOS and iNOS mRNA, total NOS enzymatic activity, and vasodilatory responses are increased by E₂ in mouse and rat aortas incubated ex vivo, the responses are blunted by 27HC (Figure 1). It was also determined that 27HC attenuates E₂

upregulation of eNOS and iNOS in cultured endothelial and VSM cells, respectively, and that the treatment of mice with 27HC, as well as with a 2% cholesterol diet, blunts vascular eNOS and iNOS mRNA expression. In addition, identical observations were made when 27HC was raised by the deletion of Cyp7b1 in mice. Furthermore, it was found that 27HC also attenuates non-nuclear signaling by both endogenous plasma membrane-associated ER in primary endothelial cells and by transfected ER α or ER β in COS-7 cells (Figure 1). The potential implications of these mechanisms on E₂-related cardiovascular protection were determined in a model of reendothelialization of the carotid artery following perivascular electric injury in mice, in which E₂ promotes reendothelialization via ER α and eNOS [28]. Whereas ovariectomized female Cyp7b1^{+/+} mice displayed enhanced reendothelialization when treated with E₂, Cyp7b1^{-/-} mice with elevated 27HC completely lacked the response to E₂. Similar effects of 27HC occurred in male mice, and the administration of 27HC to wild-type mice had the same negative impact on E₂-induced endothelial repair. These cumulative observations indicated that 27HC is a naturally-occurring SERM that inhibits the cardiovascular protective actions of E₂ [3] (Figure 2).

Impact of 27HC on Breast Cancer

Breast cancer is the most common malignancy in women other than skin cancer, with approximately one million new cases diagnosed worldwide each year [42], and postmenopausal women are particularly at increased risk of ER(+) breast cancer [43]. This risk occurs at a time when circulating estrogen levels are declining, and endocrine-based therapies against ER(+) breast cancer employing synthetic SERMs or aromatase inhibition are often ineffective or resistance develops [43], suggesting other important unknown ER-mediated mechanisms [44]. Once 27HC was identified as an endogenous SERM, and it was appreciated that its abundance increases with age, its potential actions on ER(+) breast cancer cells were tested. In MCF-7 cells, 27HC causes the recruitment of ER α to the ERE-containing region of the pS2 promoter and the upregulation of pS2 mRNA abundance, and upregulation of ER target genes regulated through AP1 sites (progesterone receptor and ERBB4) or by Sp1 elements (progesterone receptor and E2F transcription factor 1). Studies with the pure ER antagonist ICI182,780 confirmed ER dependence of these responses. Most importantly, it was shown that 27HC causes an increase in cyclin D1 expression, an increase in the number of cells entering S-phase, and an increase in cell number [18]. Thus, 27HC, which does not require aromatization, has potent impact on ER-mediated processes involved in breast cancer cell growth (Figure 2).

Impact of 27HC on Bone Mineralization

Along with its actions in the cardiovascular system and on tumor growth, estrogen plays an important role in the regulation of bone mineralization, with estrogen deficiency caused by menopause or ovariectomy resulting in pathological bone loss that can be reversed by estrogen replacement [24,45]. Recognition of the importance of estrogen in bone homeostasis and reports that hypercholesterolemia is an independent risk factor for decreased bone mass in postmenopausal women [46,47] pointed to 27HC as a potential new modifier of bone mineralization. Studies in intact female Cyp27a1^{-/-} mice deficient in 27HC revealed that while they have a greater number of trabeculae than Cyp27a1^{+/+} mice, these trabeculae are thinner. In contrast, Cyp7b1^{-/-} mice with elevated 27HC displayed a decrease in lumbar spine bone mineral density, with distal femurs with decreased bone volume/total volume fraction, decreased trabecular number and trabecular thickness, and increased trabecular separation. The administration of 27HC to wild-type female mice caused a decrease in bone mineral density and resulted in trabecular architecture similar to that observed in Cyp7b1^{-/-} mice [48]. To determine whether these observations are related to estrogen regulation of bone mineralization, additional studies were performed with E₂

treatment of intact female Cyp7b1^{-/-} mice. E₂ caused increases in BMD in the lumbar spine and in cortical bone in the null mice, and trabecular thickness and bone volume/total volume fraction also rose. In contrast, E₂ treatment of Cyp7b1^{-/-} females did not affect trabecular number and thereby trabecular separation. Ovariectomized female mice were additionally studied to mirror the condition of estrogen deprivation. In Cyp7b1^{+/+} mice, ovariectomy-induced bone loss was predictably reversed by E₂ treatment. In contrast, in Cyp7b1^{-/-} females bone mineral density was not further decreased by ovariectomy, and it was not improved by E₂. Furthermore, Cyp7b1^{-/-} females had increased urine deoxypyridinoline (DPD) compared with Cyp7b1^{+/+}, indicative of greater bone resorption, but the urine DPD levels in the null mice were not influenced by either E₂ treatment or ovariectomy [48]. These collective findings indicate that 27HC likely has adverse impact on bone mineralization (Figure 2), and that some of the effects of the oxysterol may be related to its activities as a SERM. These are important initial observations about 27HC and bone health. However, the involvement of ER in these actions of 27HC remains uncertain because the stoichiometric relationship between 27HC and estrogen in bone under the conditions studied is unknown, and ER expression or function was not manipulated directly to assess ER dependency. Additional experimentation is now warranted to better understand the actions of 27HC occurring through ER in osteoclasts, osteoblasts and other bone cells both in vitro and in vivo.

Future Directions

Nearly fifty years after the functions of the first synthetic SERM tamoxifen were delineated, the oxysterol 27HC was identified as the first endogenous SERM. Studies in mice with alterations in 27HC abundance have indicated that 27HC has adverse effects on the cardiovascular protection afforded by estrogen, and also on bone mineralization. Work in cell culture has revealed that 27HC is an endogenous ligand capable of activating breast cancer cell growth. Numerous knowledge gaps remain. First, the impact of 27HC on other ER-mediated processes, such as cognition, body weight regulation, and glucose and insulin homeostasis, warrants investigation in animal models. Second, the relationship between the abundance of 27HC and ER-mediated processes governing human health and disease should be tested using rigorous methods of 27HC quantification. Third, the potential mechanistic link that 27HC may provide between hypercholesterolemia and ER-mediated conditions requires deeper interrogation. Fourth, other nuclear and non-nuclear mechanisms of ER action are likely affected by 27HC, and these should be identified. Fifth, means of selectively modifying 27HC abundance or mechanism of action should be sought to further test its contribution to disease and to begin evaluating 27HC as a new therapeutic target. Finally, the recent discovery of 27HC as a SERM suggests that there are other endogenous ligands for ER, as well as for other steroid hormone receptors, and these should be pursued using strategies similar to those that revealed the actions of 27HC on ER. Through continuing investigation of novel endogenous steroid hormone receptor ligands such as 27HC we will gain additional understanding of this new aspect of endocrinology.

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Figure 1.

27HC attenuates nuclear and non-nuclear ER α function in endothelial cells. A. E₂ binding to nuclear ER α causes the activation of eNOS gene expression, and E₂ also activates plasma membrane-associated ER α to stimulate PI3 kinase (PI3K)- and Akt kinase-dependent activation of eNOS (activation is shown as a green arrow). These processes stimulate endothelial cell growth and migration, thereby promoting reendothelialization in vivo, and stimulate vascular relaxation. B. 27HC is an endogenous SERM that competes for ER binding to antagonize nuclear ER α induction of the eNOS gene and also ER α -dependent non-nuclear eNOS stimulation (antagonism is shown as a red block). As a result, 27HC blunts E₂-induced endothelial cell growth and migration and E₂-induced re-endothelialization and vascular relaxation.

Figure 2.

27HC has multiple actions in ER target tissues. A. In the vascular wall 27HC antagonizes both E₂-induced, ERα-dependent upregulation of eNOS in endothelium, and E₂- and ERβ-dependent upregulation of iNOS in VSM. It also blunts E₂-induced reendothelialization, which is mediated by non-nuclear ERα activation of eNOS. B. In breast cancer cells, 27HC activates ERα target genes and stimulates cell growth. C. In bone, 27HC decreases bone mineral density, potentially contributing to osteopenia. The specific roles of ER in 27HC actions on bone are yet to be determined. Activation is shown as a green arrow, and antagonism is shown as a red block.