Abstract

The discovery of a family of hormonal steroids esterified with fatty acid has raised questions concerning their physiologic role. Because of their water-insolubility these compounds are present in the circulation only as components of lipoprotein particles. Current evidence supports the hypothesis that estrogen esterification is catalyzed by lecithin:cholesterol acyltransferase associated with HDL. In addition, recent results indicate that estradiol esters are transferred from HDL to LDL particles in a cholesteryl ester transfer protein (CETP)-associated process. The studies now focus on the various possible physiologic roles proposed for these hormone derivatives, (1) functioning as fat-soluble antioxidants incorporated in lipoproteins rendering protection against oxidation of these particles, (2) providing a mechanism for hormonal storage in lipoproteins and fat tissues, (3) providing a novel hormone transport system using lipoprotein as carriers and lipoprotein receptors for entry into cells. Quantitative methods of determination of estradiol fatty acid esters in human body fluids have been developed. Preliminary studies suggest that diet-derived plant estrogens may also form fat-soluble derivatives which become incorporated in lipoproteins.

Keywords: Atherosclerosis; Hormones; Lipoproteins; Membrane transport

1. Introduction

Menstruating females demonstrate a remarkable resistance against atherosclerotic disease compared to men of similar age. Usually this has been attributed to the proposedly beneficial effects of endogenous estrogens on the plasma lipoprotein pattern. This is supported by the lower LDL-cholesterol and higher HDL-cholesterol concentrations in women compared to men, and by the fact that administration of estrogen replacement therapy to postmenopausal women lowers LDL-cholesterol and elevates HDL-cholesterol [1]. However, results from studies attempting to define changes in plasma lipoproteins brought about by endogenous estrogens have been less clearcut. Investigations concerning plasma lipoprotein patterns in hypoestrogenic amenorrheic women and those in normal menstruating females have yielded conflicting results [2,3] and it has been difficult to find consistent changes in lipoprotein lipids caused by hormonal fluctuations during the menstrual cycle [4–6]. It is plausible that, in addition to the possible role estrogen-induced changes in the lipoprotein pattern, other antiatherogenic mechanisms exist, e.g. estrogen effects on vascular reactivity, or antioxidative effects.

Human estrogens are known to exert powerful antioxidant effects in lipid–aqueous systems in vitro [7–9]. The antioxidant efficacy appears to depend on unsubstituted hydroxyl groups on the aromatic ring A of estrogen molecules [10–13], a structure similar to that seen in other natural antioxidants such as α-tocopherol and several isoflavone phytoestrogens [14] as well as flavones. The identification of δ-5-steroid and estradiol esters formed by lecithin:cholesterol acyltransferase (LCAT) [15] and the subsequent clarification of the esterification site of estradiol at C-17 in the D ring [16,17] suggested that these
derivatives might become structural components in lipoproteins. The literature concerning the family of lipophilic steroid fatty acid esters has been recently reviewed [18]. We were particularly interested in the possibility that hormonal antioxidants could make stable alterations in lipoprotein particles rendering them less susceptible to oxidation even after they have penetrated the vascular endothelium. Sequestered from the abundant water-soluble antioxidants present in the circulating blood, these lipoprotein-associated molecules would be active in the subendothelial space, possibly interfering with the atherosclerotic process. This review summarizes our recent results and discusses them in the context of related work from other laboratories.

2. Antioxidant efficacy of lipoprotein-associated estrogen fatty acid esters

In our initial studies we chemically synthesized estradiol 17-fatty acid esters and set up a system which allowed their incorporation into lipoproteins without exposing the lipoprotein particles to organic solvents used for solubilization of estrogen derivatives [19]. In this system the estradiol esters were adsorbed on the surfaces of Celite particles and the solubilizing organic solvent was evaporated to dryness. The lipoprotein was added in an aqueous buffer solution and incubated with the Celite dispersion, which resulted in transfer of estradiol esters from the particle surfaces to the lipoproteins. This made it possible to incorporate supraphysiologic amounts and to carry out oxidation experiments in a series of lipoprotein preparations containing differing amounts of estradiol esters. Such experiments utilizing Cu$^{2+}$ ions as pro-oxidants showed that enrichment of lipoprotein particles with esterified estradiol rendered them less susceptible to oxidation in vitro [19].

While it is well known that supraphysiologic concentrations of estrogen added to LDL mixtures cause inhibition of oxidation in vitro [8,9,20], there is little information concerning physiologic estrogen concentrations. Shwaery et al. [21], however, reported that in vitro incubations of physiologically relevant concentrations of estradiol with human plasma increased the oxidation resistance of LDL, and that the presence of lecithin:cholesterol acyltransferase (LCAT) was necessary for this effect. The same investigators also demonstrated that plasma incubations containing estrone did not increase the oxidation resistance of LDL although estrone was incorporated in LDL particles [22]. Estrone lacks a hydroxyl group at C-17 and was not esterified which suggested to the authors that the antioxidant efficacy was not only dependent on accumulation of estrogen in the lipoprotein but, in addition, on fatty acid esterification.

3. Antioxidant efficacy of lipoprotein-associated phytoestrogen fatty acid esters

In vitro studies have demonstrated that many isoflavone phytoestrogens exhibit antioxidant activity and that this activity is related to their structural characteristics [23–26]. Previous studies from our laboratory and others have reported that LDL particles isolated from individuals following intake of soybean phytoestrogens (mainly genistein and daidzein) were less susceptible to oxidation compared to LDLs isolated from individuals on a soy-free diet [27,28].

To explore the possible mechanisms underlying these observations, we carried out two types of experiments. First, fatty acid esters of genistein and daidzein were synthesized chemically and their incorporation in vitro in LDL was studied. Secondly, Esterbauer’s method for measuring the formation of ‘conjugated dienes’ was used in oxidation experiments of these phytoestrogen-containing LDLs with Cu$^{2+}$ as pro-oxidant. The results indicated that certain phytoestrogen esters were effectively incorporated in LDL and that some, but not all, ester-containing LDLs became resistant to oxidation [29]. Selective estrogen receptor modulators (SERMs) such as tamoxifen, which share molecular characteristics with phytoestrogens and estrogens, also exhibit inhibition of cell-mediated oxidation of LDL in vitro [30]. Currently it is not clear to what extent, if any, SERMs are incorporated in lipoproteins.

4. LCAT-induced estrogen esterification and transfer of esters from HDL to LDL

Ovarian follicular fluid has the rare characteristic that it contains one lipoprotein class, HDL, but lacks the other two major classes, VLDL and LDL. It also contains LCAT, the enzyme which esterifies cholesterol at carbon-3 and estradiol at carbon-17 [17,31]. In view of this, we started our studies concerning lipoprotein-associated estrogen by incubating labelled estradiol with follicular fluid, followed by ultracentrifugal isolation of HDL and further analysis of the radioactivity associated with this lipoprotein [32]. The results indicated that the label which had accumulated in HDL had been converted to estradiol 17-fatty acid ester, while unsubstituted estradiol had not been incorporated into the lipoprotein at all. Production and incorporation into HDL of the estradiol 17-fatty acid ester was completely inhibited by the the LCAT inhibitor dithionitrobenzoic acid (DTNB), suggesting that the process was dependent on LCAT.

Further incubations were carried out with estradiol in human plasma to investigate the interactions of estradiol esters and plasma lipoproteins. The results indicated that, after incubation, label was present not only in HDL but also in LDL, and to a small extent in VLDL [32]. As
LCAT is mainly associated with HDL, we explored the possibility that cholesteryl-ester transfer protein (CETP) might be involved in the transport of estradiol 17-esters from HDL to LDL. We designed incubation experiments in which HDL particles containing labelled estradiol 17-fatty acid esters were coincubated with native LDL obtained from the same individual. After incubation, the lipoproteins were reisolated and the radioactivity contained in them was analyzed. With increasing incubation time, a gradual shift of label from HDL to LDL occurred, and this shift was accelerated in a dose-dependent fashion by adding increasing amounts of exogenous CETP to the incubations. The results suggested somewhat unexpectedly that the same factors which regulated cholesterol esterification and transport between lipoproteins also regulated the esterification and transport of estradiol (Fig. 1) [32].

5. Quantitative determination of estradiol-17 esters in human body fluids

Only a few studies have reported quantitative determinations of endogenous estrogen ester concentrations in human tissues. Such esters have been detected in blood, muscle and fat tissue, and breast cyst fluid [33]. Very little has been published concerning plasma levels of estrogen esters since their first description by Janocko and Hochberg in 1983 [34]. In 1992 Larner et al. [33] reported circulating estradiol ester concentrations between 47.7 and 91.8 pmol/l in three women who had received gonadotropin in injections, and detected also estradiol esters in the blood of four menstruating women, although the levels were too low for accurate quantification.

We have developed a reliable method for measuring estradiol ester concentrations in early and late human pregnancy as well as in human follicular fluid [35]. Two major problems had to be overcome before the method was shown to be reproducible and accurate. First, a complete separation of the estradiol ester from unesterified estradiol had to be achieved to eliminate the possibility of contamination by the latter which was present in 100-fold higher concentrations. This was accomplished by hydrophobic chromatography on Sephadex LH-20 which allowed elution of the lipophilic ester fraction in the void volume while unesterified estradiol was retained in the column. The second problem was due to the massive contamination of the lipophilic ester fraction with plasma lipids which had to be removed to avoid interference with the assay. This was achieved by saponification hydrolysis of the ester fraction followed by a second hydrophobic chromatography on Sephadex LH-20. This time the unesterified estradiol resulting from saponification was retained in the column while all lipid contaminants were eluted. The estradiol was then eluted from the column by switching the eluting solvent to methanol [35].

The mean estradiol ester concentration in early pregnancy (first trimester) plasma was 40.4 pmol/l and that of late pregnancy (third trimester) was 404 pmol/l [35]. Although there was a 10-fold increase in the ester concentration, the ratio of esterified estradiol to unesterified estradiol did not vary much during pregnancy, 0.4–0.6%. The highest estradiol ester concentrations were obtained, in line with a previous report [17], in studies of follicular fluid, showing a mean value of 106 nmol/l, corresponding to an estradiol ester/unesterified estradiol ratio of 6%.

6. Discussion

The most important open question concerns the physiologic role of these lipophilic estrogen conjugates. Three theoretical possibilities have received most of the attention.

First, estrogen fatty esters carried in lipoproteins could provide antioxidant protection. Second, esterified estrogens, which are metabolized slowly [18], could function as an inactive hormonal reserve residing in lipoproteins and fat tissue. Third, lipoprotein-carried estrogen esters could constitute an endocrine transport system allowing entry into various cells via the lipoprotein receptors.

It is probable that most of the oxidative modification of LDL takes place in the subendothelial space in the arterial intima where it has become sequestered from the antioxidants present in plasma. Under these circumstances the antioxidant content of LDL particles may have an important protective role. α-Tocopherol, β-carotene and ubiquinone are fat-soluble endogenous antioxidants present in LDL particles, and the lipophilic drug probucol has been shown to become incorporated in LDL and to inhibit its oxidation in vitro [36]. The reports suggesting that incubations of plasma with physiologically relevant concentrations of estradiol produced LDLs with increased oxidation resistance in vitro [21,22] support the possibility that such protection could occur in vivo. If estrogen esters are produced mainly by HDL-associated LCAT, they could,
based on experiments in vitro [32] be transported from HDL to LDL in a process mediated by CETP. One can speculate that estradiol esters are positioned on the surface of LDL with the hydrophobic C-17 fatty acyl chain interspersed with the phospholipid fatty acyl chains directed towards the lipoprotein core, and the aromatic A ring with the unsubstituted C-3 hydroxyl group aligned on the lipoprotein surface. The antioxidant role of estrogen esters would appear plausible if several molecules would reside in each LDL particle, which is the case for α-tocopherol [37]. However, the studies by Schwaery et al. [21] suggested that oxidation resistance of LDL following incubation with estradiol in plasma was increased despite the fact that most of the LDL particles did not contain a single molecule of estrogen ester. This paradox needs to be further studied and accurate methods for the quantitative determination of endogenous estrogen ester concentrations in lipoproteins must be developed. Estrogen esterification, however, is only one of many phenomena which may influence LDL oxidation susceptibility. Fluctuations in plasma estradiol concentrations reportedly cause alterations in LDL particle size, composition and α-tocopherol content [38] all of which may be relevant in this respect.

Fatty acid esterification of steroid hormones is a metabolic transformation which prolongs and potentiates hormonal activity, although the esters are prohormones which require hydrolytic cleavage for their activation [18]. In theory, estrogen fatty acid esters could constitute a hormonal reservoir mainly residing in fat tissue [33]. One may speculate that specific esterases might become activated and liberate the estrogens at times when steroidogenic organs become quiescent or lose their function [18].

A further possibility is that the lipoprotein system provides an endocrine transport system enabling steroid esters to enter cells via lipoprotein receptors. This has not been studied with estrogen but Roy and Belanger [39] have suggested that LCAT-synthesized esters of pregnenolone and dehydroepiandrosterone carried in lipoproteins could serve as precursors for steroid synthesis in various tissues. The studies by this group have shown that circulating lipoprotein-associated steroid esters can be taken up into cells by lipoprotein receptors and converted into free steroids [39–41]. On the other hand, biologically active free steroids are not hydrophilic and can enter cells by diffusion through cellular membranes. This raises the question why a lipoprotein-mediated transport system would be needed at all. One possibility is that the system would make tissue-specific hormonal activity possible: esterified steroid hormones would become activated only in tissues with operating specific esterases.

Although there is mounting evidence supporting important functions for steroid esters, all of the above mentioned functions remain speculative. What is clear about this unique family of hormonal substances is that they differ from all other hormones in one respect: they circulate in the blood exclusively bound to lipoprotein particles. There are currently no reports concerning the possible effect of orally or transdermally administered estradiol on the plasma content of esterified estradiol, but such studies are in progress in our laboratory.

The isoflavone phytoestrogens present in soy protein foods share some structural characteristics with estrogens, and isoflavone-containing diets have been suggested to be protective against atherosclerosis [42]. Our finding that LDLs isolated from individuals fed with soy isoflavones showed improved oxidation resistance in vitro prompted us to investigate whether lipophilic derivatives of isoflavones could be formed and incorporated in lipoproteins. The data indicate that chemically achieved fatty acid esterification made the water-soluble isoflavones lipophilic and allowed their incorporation in LDL in vitro [29]. Preliminary incubations of labelled genistein with human plasma have suggested that lipophilic derivatives are formed and their characterization is in progress. The phytoestrogen studies were prompted by our interest in the possibilities of enriching diets with natural antiatherogenic substances. For example, dietary components that might alter the LDL in such a way that it is resistant to oxidation even when it has left the plasma compartment, are potentially important. The possible protective role of phytoestrogen derivatives is of particular interest in postmenopausal women.

References


