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Invited paper

Cholesterol oxidation products and their biological importance

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ABSTRACT

The main biological cause of oxysterols is the oxidation of cholesterol. They differ from cholesterol by the presence of additional polar groups that are typically hydroxyl, keto, hydroperoxy, epoxy, or carboxyl moieties. Under typical conditions, oxysterol concentration is maintained at a very low and precisely regulated level, with an excess of cholesterol. Like cholesterol, many oxysterols are hydrophobic and hence confined to cell membranes. However, small chemical differences between the sterols can significantly affect how they interact with other membrane components, and this in turn can have a substantial effect on membrane properties. In this spirit, this review describes the biological importance and the roles of oxysterols in the human body. We focus primarily on the effect of oxysterols on lipid membranes, but we also consider other issues such as enzymatic and nonenzymatic synthesis processes of oxysterols as well as pathological conditions induced by oxysterols.

1. Introduction

The function of living systems is inherently based on cells, which are the key building blocks of living organisms. Each cell in a body is surrounded by a cell membrane, which essentially is a functional interface separating the cell from its surroundings. Moreover, most internal cell organelles and structures are also surrounded by a membrane. Mammalian cell membranes host a substantial amount of proteins found in cells (Niemela et al., 2009; van Meer et al., 2008). They are built of a lipid bilayer composed primarily of phospholipids and cholesterol. Cholesterol is particularly abundant in the plasma membranes, representing typically 25–40 mol% of the total lipid content (Ikonen, 2008; Ikonen and Jansen, 2008; Simons and Sampaio, 2011). In internal cell membranes the cholesterol content is typically lower than in the plasma membrane (Coskun and Simons, 2011; Sezgin et al., 2012).

Cholesterol regulates a plethora of biological processes, either by directly interacting with proteins embedded in the membranes or by regulating the biophysical properties of lipid bilayers and, hence, indirectly modulating protein function. The flat and stiff steroid moiety present in the cholesterol molecule implies that the conformational order of phospholipids in the vicinity of cholesterol is promoted extensively, thereby increasing lipid

Abbreviations: chol, cholesterol; 7α/β-OOH-chol, 7α/β-hydroperoxycholesterol; 7α/β-OH-chol, 7α/β-hydroxycholesterol; 7-Keto-chol, 7-ketocholesterol; 24S-OH-chol, 24S-hydroxycholesterol; 27-OH-chol, 27-hydroxycholesterol; 4β-OH-chol, 4β-hydroxycholesterol; 25-OH-chol, 25-hydroxycholesterol; 5-COOH-chol, 3β-hydroxy-5-cholestenoic acid; 24S,25-Epoxy-chol, 24S,25-epoxycholesterol; 27-OH-7-Keto-chol, 27-hydroxy-7-ketocholesterol; 3β-OH-COOH-chol, 3β-hydroxy-5-cholestenoic acid; 7-Keto-chol-3-sulf, 7-ketocholesterol-3-sulfate; 5α/β,6α/β-Epoxy-chol, 5α/β,6α/β-epoxycholesterol; 26-OH-chol, 26-hydroxycholesterol; 22-OH-chol, 22-hydroxycholesterol; 20α/β-OH-chol, 20α/β-hydroxycholesterol; 19-OH-chol, 19-hydroxycholesterol; 4β-OH-chol, 4β-hydroxycholesterol; 5-OH-chol, 5-hydroxycholesterol; 3α/β,5α/β,6α/β-3OH-chol, 3α/β,5α/β,6α/β-trihydroxycholestane; 3β-OH-chol, 3β-hydroxycholesterol; 22-Keto-chol, 22-ketocholeterol; cholestenone, 4-cholesten-3-one; 7α,25-2OH-chol, 7α,25-dihydroxycholesterol; CH25H, cholesterol 25-hydroxylase; ER, endoplasmic reticulum; ROS, reactive oxygen species; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LXR, liver X receptor; SREBPs, sterol regulatory element-binding protein; Insig, Insulin-induced gene; EBI2, Epstein-Barr virus-induced gene 2; BBB, blood–brain barrier; DPH, diphenylhexatriene; TDFS, time-dependent fluorescence shift; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; DRM, detergent-resistant membrane; AD, , Alzheimer's disease; Aβ, amyloid beta; PD, Parkinson's disease; HD, Huntington's disease; MS, multiple sclerosis; AMD, age-related macular degeneration; RPE, retinal pigment epithelium.

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packing, decreasing membrane elasticity, and rendering the membrane less accessible to small water-soluble molecules (Berkowitz, 2009; Epand and Bottega, 1987; Kučerka et al., 2007; Martinez et al., 2002; Pandit and Scott, 2009; Rog et al., 2009). Cholesterol is a precursor in bile acids and steroid hormones' synthesis (Chiang, 2004), participates in lipid nanodomains' formation (Edidin, 2003; Incardona and Eaton, 2000; Simons and Ikonen, 2000; Simons and Sampaio, 2011), and regulates protein functions (Epand, 2006; Kuwabara and Labouesse, 2002).

Cholesterol molecules present in lipid bilayers are largely susceptible to oxidation. Cholesterol oxidation products known as oxysterols, as well as phytosterol oxidation products also considered to be oxysterols (Guardiola et al., 2002), differ from cholesterol by the presence of one or more oxygen-containing groups not found in cholesterol. Such a relatively minor change in the chemical structure of cholesterol leads to significant changes in the biophysical properties of oxysterols (Kulig et al., 2015b; Massey, 2006; Massey and Pownall, 2006; Olkkonen and Hynynen, 2009), which in turn may significantly modulate the properties and the dynamics of the lipid bilayers.

In this review, we will address the synthesis of oxysterols during cholesterol oxidation (Section 2) as well as their typical levels in a human body (Section 3). Further, we also discuss the biological functions of oxysterols (Section 4) and their effects on the properties of lipid bilayers (Section 5). Finally, we consider human pathological conditions and diseases, whose cause is associated with the effects of oxysterols (Section 6).

2. Formation of oxysterols

The main biological source of oxysterols is cholesterol, which, via numerous chemical reactions, is transformed into its oxidized derivatives. These reactions are either nonenzymatic or enzymatic. Nonenzymatic cholesterol oxidation leads mainly to the generation of products in which the sterol ring system is oxidized. On the other hand, enzymatic processes usually end up in products with an oxidized side chain. However, there are a few exceptions to this rule. Nonetheless, in general oxysterols differ from cholesterol by additional polar groups (one or several); these are typically hydroxyl, keto, hydroperoxy, epoxy, or carboxyl moieties. The main biologically relevant oxysterols, including their full and abbreviated names, are presented in Table S1 (Supplementary Material). In this context, we wish to stress that the naming convention of oxysterols in the field is not unique and may cause confusion (see, e.g., controversy on the naming of 27- hydroxycholesterol (Fakheri and Javitt, 2012)). Therefore, it is a good practice to publish the oxysterol structure whenever possible when those are discussed in the literature.

2.1. As oxysterols are being born, how and where does it take place?

Nonenzymatic cholesterol oxidation occurs due to reactions with reactive oxygen species (ROS) that are physiologically present in a body (Brown and Jessup, 2009). In this respect, the oxidation of cholesterol is similar to that of other lipids that are also prone to attacks by ROS (Fruhwirth et al., 2007; Jurkiewicz et al., 2012). The interaction of cholesterol with ROS leads to the abstraction of hydrogen from the C-7 position (see Table S1) and the formation of a radical carbon (Brown and Jessup, 2009). This long-living radical can react with oxygen, thus forming a cholesterol peroxyl radical ($COO^{\bullet}$). This radical can further abstract hydrogen from other lipid molecules, leading to the formation of a relatively stable cholesterol hydroperoxide (7α- or 7β-OOH-chol) with the —OOH moiety at the C-7 position. Although the 7-OOH-chol species are the major nonenzymatic cholesterol oxidation products, their tissue levels are relatively low due to their further transformation into hydroxy- and ketocholesterols (7α- and 7β-OH-chol, and 7-Keto-chol, respectively), which are the most abundant nonenzymatically generated oxysterols present in most tissues (Brown and Jessup, 1999, 2009; Brown et al., 1997).

Enzymatic oxidation of cholesterol occurs due to the action of several enzymes (Olkkonen et al., 2012; Russell, 2000), mostly related to the cytochrome P450 family. Among them, CYP46A1 catalyzes the formation of 24S-OH-chol (Björkhem, 2007). This enzyme is present mostly in the endoplasmic reticulum of neural cells and the retina (Bjorkhem et al., 1998; Bretillon et al., 2007). Two enzymes, CYP7A1 and CYP27A1, take part in the synthesis of 7α-OH-chol and 27-OH-chol, occurring predominantly in the liver (Bjorkhem and Eggertsen, 2001). CYP3A4, involved mainly in drug metabolism in the liver, catalyzes the formation of 4β-OH-chol (Bodin et al., 2002). Cholesterol 25-hydroxylase (CH25H), which is a nonheme-iron-containing enzyme present in endoplasmic reticulum (ER) and the Golgi of cells in most tissues, catalyzes the formation of 25-OH-chol (Russell, 2000). Among the physiologically relevant oxysterols, 24S,25-Epoxy-chol has a somewhat distinctive origin because it is generated enzymatically as a side product of cholesterol biosynthesis (Nelson et al., 1980, 1981).

It should be noted that there is also an exogenous source of oxysterols in the form of dietary intake (Otaegui-Arrazola et al., 2010). Oxidized sterols occur in elevated levels in cholesterol-rich food due to oxidative conditions connected with food production, storage, and preparation (Brown and Jessup, 2009). It has been demonstrated that oxysterols absorbed from food can enter circulation (Brown and Jessup, 1999; Carpenter, 2002). However, an exact quantitative estimation of the levels of endogenous vs. exogenous oxysterols is missing.

As oxysterols originate from cholesterol, their generation depends, to a certain extent, on the presence of cholesterol. It can be roughly estimated that under physiological conditions the concentration of oxysterols is three orders of magnitude lower than that of cholesterol (for a detailed discussion of oxysterol concentration, see the next Section) (Brown and Jessup, 2009). Note also that under oxidative stress in the presence of ROS cholesterol is less prone to nonenzymatic oxidation than unsaturated acyl chains of phospholipids, the latter usually being in abundance with respect to cholesterol (Noguchi et al., 1998). Nevertheless, *in vitro* studies have shown that oxysterols are more abundant than other oxidized lipids in the cell membranes of oxidatively stressed cells, which is probably due to less efficient mechanisms of oxysterol clearance (Brown and Jessup, 2009; Saito et al., 2007). Regarding the clearance mechanisms, oxysterols are eliminated by either metabolic processes or direct elimination. Metabolism leads to the formation of chemically modified species that differ in their physicochemical properties (e.g., solubility or lipophilicity) and/or physiological action (e.g., cytotoxicity) from parent oxysterols.

2.2. Oxysterols' metabolism and elimination

There are four major routes of oxysterol metabolism (Gill et al., 2008). First, oxysterols can be esterified by cholesterol acyltransferases (ACAT and LCAT). Second, CYP27A1, involved in oxysterol formation, can also catalyze further oxidation of ring-oxidized sterols to higher oxidized oxysterols (e.g., 7-Keto-chol to 27-OH-7-Keto-chol, or 27-OH-chol to 3β-OH-5-COOH-chol acid), which in turn can be effectively transformed into more water-soluble species (Brown et al., 2000b). Third, 11β-hydroxysteroid dehydrogenase can reduce oxidized sterols (e.g., 7-Keto-chol to 7β-OH-chol) (Jessup and Brown, 2005). Fourth, the cholesterol sulfotransferase enzyme can sulfate both ring and side-chain oxysterols