Acta Medica 2014; 3: 75-79

acta medica

Review Article

Oxysterols and Their Metabolic Roles Beyond Cholesterol: A Reappraisal

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Received 29 August 2014, accepted 10 October 2014, published online 4 November 2014

ABSTRACT

Oxysterols are oxidized products of cholesterol metabolism that are found in trace amounts in the biological system and play important roles in the body. It has been shown that they are involved in cholesterol homeostasis, neurogenesis, protein prenylation and in the functioning of the immune system. Oxysterols are also implicated in the aetiology of disease states including atherosclerosis, neurodegenerative and inflammatory diseases due to their cytotoxic and proapoptotic properties. Their functions have not been fully understood, yet. In this review, general properties of oxysterols and their relationship with the pathogenesis of some major diseases have been summarized.

Key words: cholesterol, oxysterols, lipid metabolism, oxysterol receptor

Introduction

Sterol is a particular type of lipid that consists of four fused carbon rings with a hydroxyl group at the end. There are various sterol species in human plasma. Cholesterol is the most abundant sterol, making up almost 99% of all the sterols in circulation. Sterols of plant origin like campesterol and sitosterol may be also found in plasma [1].

Cholesterol is one of the major components of mammalian cell membrane and regulates membrane fluidity (Figure 1). Cholesterol rich lipid rafts in the membrane perform critical roles during cellular proliferation, signaling and differentiation [2-4]. Synthesis of cholesterol is mediated by a biosynthetic pathway with several enzymatic steps (Figure 2) [5-8]. Various metabolites occur during the pathway of cholesterol metabolism and control different biological processes. Cholesterol is converted to more polar compounds by the addition of oxygen containing groups such as epoxy, hydroxy or ketone. These 27-carbon intermediates or end products of cholesterol are called oxysterols. These compounds have biochemical reactivity and because of additional oxygen, they can cross the lipophilic membranes more easily [9-13]. Oxysterols can be formed in the body by enzymatically or non-enzymatically driven reactions. Most of the enzymatic formations of oxysterol species are regulated by the cytocrome p450 (CYP) enzyme family in the mitochondria and endoplasmic reticulum [12, 14, 15]. They are transported by lipoproteins in the circulation in a similar manner to cholesterol [10].

Oxysterols are produced in mammalian cells, hepatic or extrahepatic tissues, and blood at lower quantities under normal physiologic activities. However, oxysterol concentrations increase in pathologic conditions such as atherosclerosis, inflammation or cancer [12, 13, 15]. It has been shown that oxysterol production stimulates foam cell formation and the development of atherosclerotic plaques [16].

Oxysterols are also found in foods originated from animal fat and can also be generated while food storing or cooking [13]. We focus on general properties of oxysterols and their relationship with the pathogenesis of some major diseases in this review.

General Characteristics of Oxysterols

The term oxysterol denotes a group of molecules that are oxygenated metabolites of cholesterol or by-products of the cholesterol metabolism in the body [17]. Addition of oxygen decreases the half-life

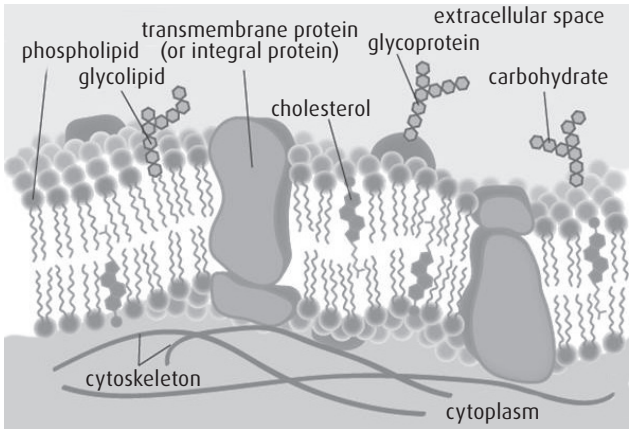

Figure 1. Location of the cholesterol on cell membrane

of the cholesterol molecule drastically and directs it either to the excretory pathway or to bile acid formation via further oxidation. Thus, formation of oxysterols can be regarded as a way to route the cholesterol molecule for catabolism [11, 12].

Uptake of cholesterol and/or oxysterols is achieved in two ways that includes consumption of food or excretion by the liver into the bile, which is absorbed by enterocytes in the intestine [18].

Oxysterols are more lipophilic than cholesterol and easily pass biomembranes and the blood brain barrier [19-21]. Hydroxy, keto, hydroperoxy, epoxy, and carboxyl moieties are the most common oxidative modifications of cholesterol [22]. The dominating oxysterol in the circulation, 7-α-hydroxycholesterol, comes from the cholesterol oxidation by 7-α hydroxylase in the liver during bile acid synthesis [23].

As mentioned above oxysterols can be produced by enzymatic or non-enzymatic reactions. Enzymatic pathways involve CYP450 family enzymes. On the other hand, non-enzymatic reaction (or auto-oxidation) involves reactive oxygen and nitrogen species

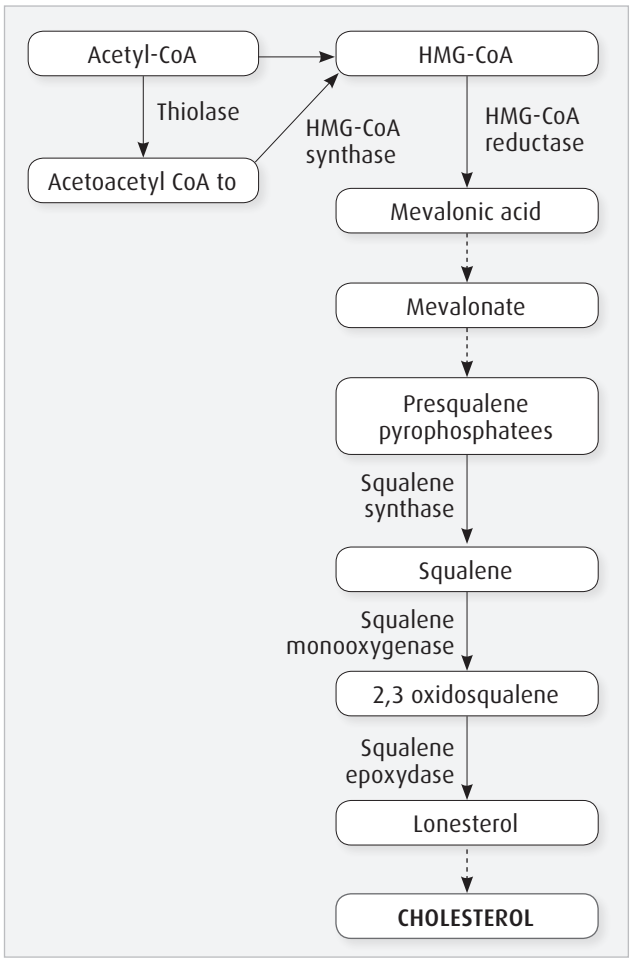

Figure 2. Pathway of cholesterol biosynthesis

(ROS, RNS) [15, 16, 18]. Mitochondria and endoplasmic reticulum are two organelles producing the physiologically important oxysterols that are synthesized by cholesterol hydroxylases belonging to the CYP450 family [17, 23]. 27-, 24 (S) -, 7-α-, and 4-β-hydroxycholesterol are the most abundant of these oxysterols and are found in human serum [9, 12, 21, 24]. Common oxysterol derivatives are shown in figure 3.

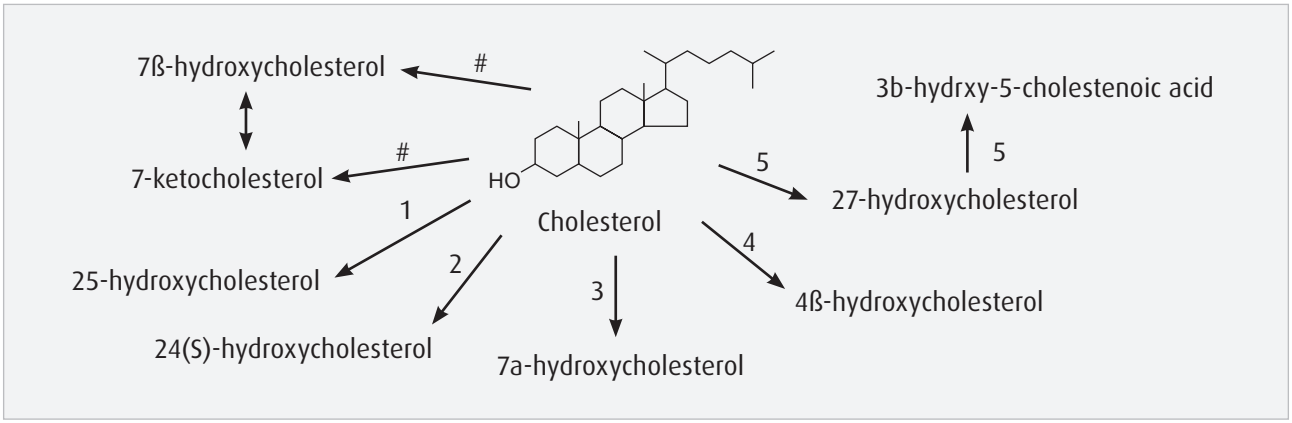

Figure 3. Formation of common oxysterols from cholesterol via *cytochrome* (CYP) enzymes or autooxidation. # Autooxidation (1) CYP25H; (2) CYP46A1; (3) CYP7A1;

Oxysterols also arise in vivo or during food processing through non-enzymatic, free radical, lipid peroxide, or divalent cation-induced oxidative processes, often termed as cholesterol autoxidation [9, 12, 20]. The most abundant oxysterols generated through autoxidation are modified at the 7-position of the cholesterol B-ring. These include 7-ketocholesterol and 7-β-hydroxycholesterol and 7-oxo-cholesterol with prominent cytotoxic and pro-apoptotic properties [25]. The major oxysterols in the circulation are distributed by lipoproteins. To illustrate, 7-hydroxycholesterol, 27-hydroxycholesterol, 24(S)-hydroxycholesterol, 4-β-hydroxycholesterol are quantitatively important oxysterol derivatives [12, 20].

Physiological Roles of Oxysterols in the Body

Besides being intermediates in cholesterol degradation, oxysterols are a class of potent regulatory molecules, however, their role in physiology is still not clear. Although oxysterols are found in trace amounts in the biological systems, they behave as ligands to nuclear receptors; therefore their metabolic roles have been under investigation [2, 9, 12, 26]. Lipid-protein interactions are very important for the cellular activities of oxysterols particularly in the regulation of key proteins in the cholesterol metabolism [9].

Physiologic roles of steroid hormones in the body are well known. Oxysterols have steroidal structures and regulatory effects on cellular membrane interactions via receptor-ligand binding, activation of signal transduction and morphogenesis. These interactions can cause apoptosis induction as well as cytotoxicity [10, 18]. In general, oxysterols' actions can be divided into three different categories that include LXR membrane dynamics, protein interactions, and nuclear receptor binding [27]. Biological activities and major functions of oxysterols are shown in figure 4 [28].

Oxysterols bind and activate different nuclear receptors such as related orphan receptors, farnesoid X receptors, estrogen receptors as well as liver X receptors (LXR) [27]. Enzymatically generated oxysterols are indicated to play a role in the regulation of cholesterol metabolism [10] and act as important modulators of insulin by acting as agonists of LXRs [8, 10]. LXRs are members of a nuclear receptor family of transcription factors that regulate cholesterol homeostasis and protect the cells from excess cellular cholesterol production. These receptors also arrange cholesterol transport and efflux [5, 18-29]. Oxysterols are mediators in cholesterol homeostasis and regulate this pathway by different proteins and modulators of hedgehog signaling [18].

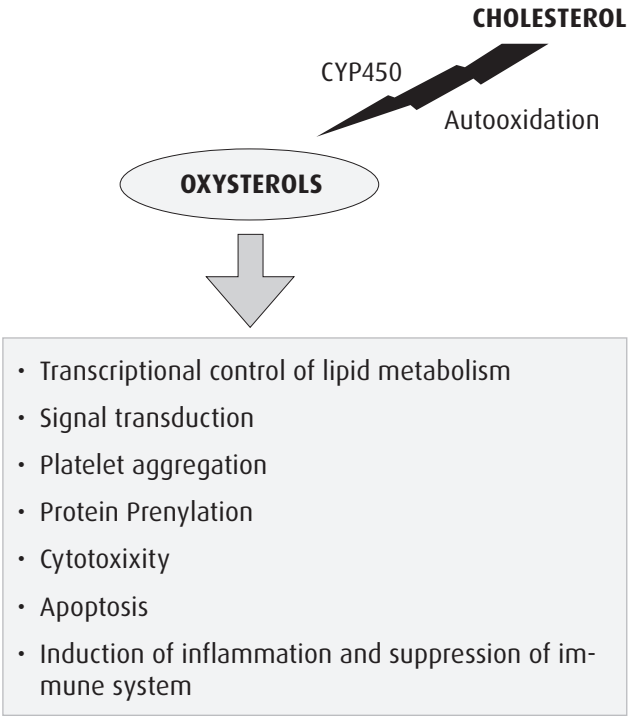

Figure 4. Some of the major pathologic and physiological functions of oxysterols

Oxysterols also regulate the functioning of the immune system [17]. To illustrate, 7α,25-dihydroxycholesterol is a selective agonist, an important receptor of immune system [18].

The innate immune system regulates adaptive immunity via oxysterols. Macrophage derived oxysterols suppress IgA production by B cells. Upon activation of their toll-like receptors, macrophages also produce 25-hydroxycholesterol that is found in trace amounts in normal plasma [10, 13].

27-hydroxycholesterol is important for the daily elimination of cholesterol from the body. It takes part in the alternate pathway of bile acid synthesis and reverse cholesterol transport by extrahepatic tissue. 7-oxo, 7-α-hydroxy and 7-β-hydroxy cholesterol are formed by autoxidation of cholesterol. 7-*α*-hydroxy cholesterol can be used as a marker of bile acid synthesis in the body [25]. 7-β-hydroxy cholesterol might reflect oxidative stress in vivo [30]. 24(S)-hydroxy-cholesterol is almost totally produced in the brain and given to the circulation via the blood brain barrier. This is a marker for the turnover of cholesterol in the brain [31].

Oxysterol binding proteins are cytoplasmic proteins that bind oxygenated derivatives of cholesterol and function as receptors for endogenous regulatory oxysterols [32]. Oxysterol binding proteins are totally different from sterol carrier proteins [33]. Although functional roles of oxysterol binding proteins have not been totally understood, they possibly function in the sterol signaling and/or transport. Oxysterol binding proteins modulate sterol-dependent regulation of ceramide transport and sphingomyelin synthesis by travelling between the endoplasmic reticulum and golgi apparatus [34].

Association between oxysterols and different pathologies including cancer

Oxysterols are also implicated in the aetiology of disease states including atherosclerosis, neurodegenerative and inflammatory diseases [17].

The biosynthetic cholesterol pathway must be balanced and supported by other pathways that enable cholesterol to be discarded. Although, cholesterol is necessary for cellular functions at a particular level, excess cellular cholesterol is harmful [5]. Oxysterols are the products of cholesterol elimination and regarded as physiological mediators in a number of cholesterol-mediated pathologic conditions such as atherosclerosis, apoptosis, necrosis, inflammation, immunosuppression, neurodegeneration and development of gallstones [9, 10, 13, 18, 35].

As mentioned in the previous section, oxysterols have important roles as regulators in the lipid metabolism as well as differentiation, developmental and inflammatory processes. Oxysterols have cytotoxic and proapoptotic activity which cause diseases. Oxysterol levels are increased in pathophysiologic conditions such as macrophage foam cells, atherosclerotic lesions, cataracts, osteoporosis, multiple sclerosis, and Alzheimer's disease [13, 18]. The cytotoxic potential of oxysterols can contribute to the disease processes. For example 27-hydroxycholesterol that is specifically produced by nerve cells has been investigated as a potential biomarker for neurodegenerative disorders such as Alzheimer's disease [12, 18, 35].

Oxysterols enhance the inflammatory reactions by stimulating synthesis of cytokines, tumor necrosis factor α (TNF-α), interleukins and other inflammatory proteins that causes the progression of many chronic diseases [13].

It has been proven that there is an association between plasma cholesterol and atherosclerosis [10]. Lipid oxidation products like oxysterols may cause endothelial cell dysfunction, atherosclerotic plaque formation, apoptosis and necrosis [9, 13]. Forming oxysterol by altered cholesterol metabolism deteriorate cholesterol distribution in the brain and can also cause Alzheimer's and Parkinson's diseases and other neurodegenerative disorders [13, 21].

It has been shown that a cholesterol rich diet can worsen inflammatory bowel disease by increasing inflammation and apoptosis that are mediated by oxysterol signaling [13]. On the other hand, cytokines in retina cells can be increased by oxysterols and this inflammatory response leads to age related macular degeneration [36].

Oxysterols can interfere with cell proliferation by controlling the transcription of the key proteins, in cholesterol synthesis. Several reports described the relation between oxysterol and several types of cancers such as lung, colon or cholangiocarcinogenesis. Additionally, oxysterols have mutagenic effects by damaging DNA, and may play a key role in the different stages of carcinogenesis. For example, oxysterols may take part in the initiation step via increased ROS and RNS or tumor promotion can be accelerated by oxysterols. On the other hand oxysterols also generate the cellular response in tumor tissue through LXRs that activate target genes in the inflammatory process [26, 35, 37-39].

Conclusion

Oxysterols are very important compounds because of their biologic and pathologic effects. The accumulation of oxysterols can induce apoptosis, necrosis and cytokine production. Many *in vivo* and *in vitro* studies have demonstrated that there is a link between different types of oxysterols and chronic inflammatory diseases and various kinds of cancers. They play an important role in different stages of the disease formation.

It is important to underline that oxysterol studies have difficulties whether they present exogenously or endogenously. However, further studies are necessary to understand the mechanism of action of oxysterols and developing treatment approachs of the diseases.

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