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Lumisterol is metabolized by CYP11A1: Discovery of a new pathway

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ABSTRACT

Lumisterol3 (L3) is produced by photochemical transformation of 7-dehydrocholesterol (7-DHC) during exposure to high doses of ultraviolet B radiation. It has been assumed that L3 is biologically inactive and is not metabolized in the body. However, some synthetic derivatives of L3 display biological activity. The aim of this study was to test the ability of CYP11A1 to metabolize L3. Incubation of L3 with bovine or human CYP11A1 resulted in the formation of three major and a number of minor products. The catalytic efficiency of bovine CYP11A1 for metabolism of L3 dissolved in 2-hydroxypropyl-β-cyclodextrin was approximately 20% of that reported for vitamin D3 and cholesterol. The structures of the three major products were identified as 24-hydroxy-L3, 22-hydroxy-L3 and 20,22-dihydroxy-L3 by NMR. 22-Hydroxy-L3 was further metabolized by bovine CYP11A1 to 20,22-dihydroxy-L3. Both 22-hydroxy-L3 and 20,22-dihydroxy-L3 gave rise to a minor metabolite identified from authentic standard and mass spectrometry as pregnalumisterol (pL) (product of C20–C22 side chain cleavage of L3) and two trihydroxy-L3 products. The capability of tissues expressing CYP11A1 to metabolize L3 was demonstrated using pig adrenal fragments where 20,22-dihydroxy-L3, 22-hydroxy-L3, 24-hydroxy-L3 and pL were detected by LC/MS. Thus, we have established that L3 is metabolized by CYP11A1 to 22- and 24-hydroxy-L3 and 20,22-dihydroxy-L3 as major products, as well as to pL and other minor products. The previously reported biological activity of pL and the presence of CYP11A1 in skin suggest that this pathway may serve to produce biologically active products from L3, emphasizing a novel role of CYP11A1 in sterol metabolism.

1. Introduction

Vitamin D3 is produced by the action of UVB radiation (280–320 nm spectrum of solar light) on 7-dehydrocholesterol (7-DHC) in the skin (Holick, 2003; Holick et al., 1980; MacLaughlin et al., 1982). The initial event is the photochemical breaking of the C9–C10 bond in the B ring of 7-DHC resulting in the formation of previtamin D3 (Fig. 1). Once formed, previtamin D3 undergoes thermal isomerization in the skin over several hours to form vitamin D3. With further exposure to UVB radiation, previtamin D3 undergoes photoisomerization to lumisterol3 (L3) and tachysterol3 (T3) (Fig. 1) (Holick, 2003; Wacker and Holick, 2013). These photochemical reactions are reversible and are dependent on the temperature and UVB dose. T3 is the most photoreactive of the three isomers and sunlight drives the conversion of T3 to L3 *via* previtamin D3, resulting in L3 being the major photoisomer observed in human skin after prolonged UVB exposure (Holick et al., 1981; MacLaughlin et al., 1982). The conversion of previtamin D3 to L3 involves reformation of the C9–C10 bond but in a 9β,10α-configuration, making it a stereoisomer of 7-DHC.

CYP11A1, also known as cytochrome P450scc, catalyzes the first step in steroid hormone synthesis, the cleavage of the side chain of cholesterol to produce pregnenolone. This reaction involves initial hydroxylation of the cholesterol side chain in the 22*R* position, hydroxylation at C20 and then scission of the side chain between C20 and C22 (Tuckey, 2005). We and others have shown that

Abbreviations: cyclodextrin, 2-hydroxypropyl-β-cyclodextrin; 7-DHC, 7-dehydrocholesterol; L3, lumisterol3; 20(OH)L3, 20-hydroxylumisterol3; 22(OH)L3, 22-hydroxylumisterol3; 24(OH)L3, 24-hydroxylumisterol3; 20,22(OH)2L3, 20,22-dihydroxylumisterol3; pL, pregnalumisterol; RT, retention time; T3, tachysterol3.

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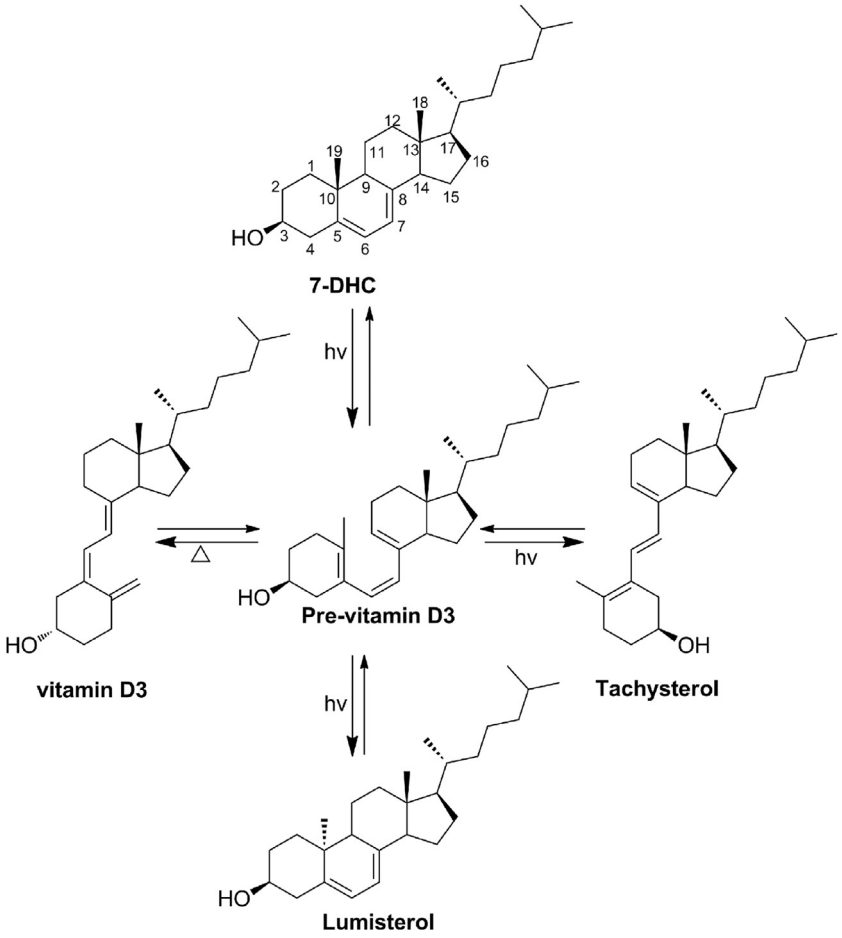

Fig. 1. Photosynthesis of previtamin D3, tachysterol and lumisterol.

CYP11A1 can also cleave the side chain of 7-DHC, and can hydroxylate the side chain of vitamin D3, vitamin D2 and ergosterol, producing a number of biologically active derivatives (reviewed in Slominski et al., 2014a). Guryev et al. (2003) and Strushkevich et al. (2011) reported that CYP11A1 hydroxylates vitamin D3 at C20 and C22 producing 20-hydroxyvitamin D3 and 20,22-dihydroxyvitamin D3 as major products. We also demonstrated the ability of CYP11A1 to hydroxylate vitamin D3 at these positions and further showed that C23 is a major site of hydroxylation with 20,23-dihydroxyvitamin D3 being the next most abundant product after 20-hydroxyvitamin D3 (Slominski et al., 2005a; Tuckey et al., 2008a, 2008b, 2011). Importantly, we have provided initial evidence that CYP11A1-initiated metabolism of 7-DHC, vitamin D3 and vitamin D2 can occur under *in vivo* conditions (Slominski et al., 2009, 2012a,b, 2014a,b). The novel CYP11A1-derived hydroxy-derivatives of vitamin D3 are active in inhibiting proliferation and stimulating differentiation in a range of cell types cultured *in vitro* (reviewed in Slominski et al., 2014a), and in reducing inflammation and skin fibrosis in mice *in vivo* (Slominski et al., 2013, 2014a), but lack the toxic and calcemic effects seen with high doses of 1,25$(OH)_2$D3 (Slominski et al., 2010; Wang et al., 2012). We and others have also reported the presence of CYP11A1 in human skin (Slominski et al., 1996, 2004, 2014a; Thiboutot et al., 2003) and have detected CYP11A1-dependent production of 20(OH)D3 by cultured keratinocytes in the absence of exogenous vitamin D3 substrate (Slominski et al., 2012a). These findings, together with the ability of CYP11A1 to act on 7-DHC (Slominski et al., 2004, 2009, 2012b), ergosterol (Slominski et al., 2005b; Tuckey et al., 2012) and vitamin D2 (Nguyen et al., 2009; Slominski et al., 2006, 2014b) has prompted us to test the ability of this enzyme to act on L3.

2. Materials and methods

2.1. Materials

2-Hydroxypropyl-β-cyclodextrin (cyclodextrin), vitamin D3, dioleoyl phosphatidylcholine, bovine heart cardiolipin and NADPH were from Sigma–Aldrich Pty. Ltd. (Sydney, Australia). L3 was from Toronto Research Chemicals (North York, Canada). Prior to use it was purified by HPLC on a C18 column (Grace Alltima, 25 cm × 4.6 mm, particle size 5 μm) using a 64–100% methanol in water gradient for 15 min followed by 100% methanol for 50 min, at a flow rate of 0.5 mL/min. Pregnalumisterol (pL) and 20-hydroxylumisterol3 (20(OH)L3) were synthesized by UVB irradiation of 7-dehydropregnenolone and 20-hydroxy-7-DHC, respectively, purified and their structure confirmed by NMR as described previously (Zmijewski et al., 2008).

2.2. Preparation of enzymes

Bovine and human CYP11A1, human adrenodoxin and human adrenodoxin reductase were expressed in *Escherichia coli* and purified as described previously (Tuckey and Sadleir, 1999; Tuckey et al., 2012; Woods et al., 1998). Highly purified bovine CYP11A1 was used for small scale metabolic and kinetic studies and was prepared from adrenal mitochondria by extraction with sodium