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Surprising Inability of Singlet Oxygen-Generated 6-Hydroperoxycholesterol to Induce Damaging Free Radical Lipid Peroxidation in Cell Membranes[†]

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Abstract

Singlet oxygen attack on cholesterol (Ch), a prominent monounsaturated lipid of mammalian cell plasma membranes, gives rise to three hydroperoxide (ChOOH) isomers, 5 α -OOH, 6 α -OOH and 6 β -OOH, the latter two in lower yield than 5 α -OOH, and 6 α -OOH in lowest yield. A third possible positional isomer, 7 α -OOH and 7 β -OOH, is produced by free radical attack. In the presence of iron and ascorbate (Fe/AH), 5 α -OOH or 6 β -OOH in phosphatidylcholine/Ch/ChOOH (20:15:1 by mol) liposomes was reduced to its corresponding alcohol, the rate constant being approximately the same for both ChOOHs. Using [¹⁴C]Ch as an *in situ* probe, we found that liposomal 5 α -OOH readily set off free radical-mediated (chain) peroxidation reactions when exposed to Fe/AH, whereas 6 β -OOH under the same conditions did not. Moreover, liposomal 5 α -OOH triggered robust chain peroxidation in [¹⁴C]Ch-labeled L1210 cells, leading to cell death, whereas 6 β -OOH was essentially inert in this regard. Thus, 5 α -OOH and 6 β -OOH undergo iron-catalyzed reductive turnover, but only the former can provoke toxic membrane damage. These novel findings have important implications for UVA-induced photodamage in Ch-rich tissues like skin and eye, where ¹O₂ often plays a major role.

Keywords

Photosensitization; Singlet Oxygen; Cholesterol; Cholesterol Hydroperoxides; Cell Membranes; Lipid Peroxidation

Introduction

At least 80 % of the cholesterol (Ch) of most mature mammalian cells is located in the plasma membrane, where it comprises ~45 mol % of the total lipid (1,2). Like other unsaturated lipids, Ch is subject to membrane-damaging oxidative modification under conditions of oxidative stress, including photodynamic stress (2,3). Among the many different products/intermediates generated by Ch (photo)oxidation, hydroperoxide species (ChOOHs) are of great interest not only because of their importance as mechanistic indicators, but also because of their diverse fates, e.g. damage-expanding one-electron

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reduction catalyzed by iron, damage-attenuating two-electron reduction catalyzed by selenoperoxidase GPx4, and delocalized redox signaling via intermembrane translocation (3-6). In singlet oxygen ($^1\text{O}_2$)-mediated Ch photooxidation (Type II chemistry), three primary ChOOHs are generated, 3 β -hydroxy-5 α -cholest-6-ene-5-hydroperoxide (5 α -OOH), 3 β -hydroxycholest-4-ene-6 α -hydroperoxide (6 α -OOH), and 3 β -hydroxycholest-4-ene-6 β -hydroperoxide (6 β -OOH), each of which arises via ene-addition of $^1\text{O}_2$ (7,8). The observed accumulation rates of these hydroperoxides during photooxidation of Ch in solution, liposomal membranes, or cells varies as follows: 5 α -OOH $\gg$ 6 β -OOH > 6 α -OOH (5,8). An earlier study provided rational explanations for this trend based on steric factors in the Ch A/B rings (5). In free radical-mediated Ch photooxidation (Type I chemistry), as in ordinary autoxidation, the most prominent ChOOHs formed are 3 β -hydroxycholest-5-ene-7 α -hydroperoxide (7 α -OOH) and 3 β -hydroxycholest-5-ene-7 β -hydroperoxide (7 β -OOH) (9). These species, along with end-products such as 5,6-epoxide, 7-ketone, and the diols 7 α -OH and 7 β -OH, also appear when $^1\text{O}_2$ -derived 5 α -OOH undergoes free radical chain-initiating one-electron reduction in membranes (4,5). When comparing the ability of liposomal 5 α -OOH and 6 β -OOH to trigger such reactions upon exposure to a lipophilic iron chelate ($\text{Fe}(\text{HQ})_3$) and ascorbate (AH^-), we found that both peroxides underwent reductive decay at essentially the same rate (5). In the process, 5 α -OOH induced robust chain peroxidation of other liposomal lipids. Surprisingly, however, 6 β -OOH produced little, if any, such effect. Consistently, 5 α -OOH triggered lethal chain peroxidation of plasma membrane lipids in L1210 cells, whereas 6 β -OOH did not. These novel findings, along with possible explanations for the observed differences, are reported for the first time in this paper.

Materials and Methods

General materials

Unlabeled Ch, Chelex-100, 8-hydroxyquinoline, ascorbic acid, RPMI 1640 growth medium, and fetal bovine serum were obtained from Sigma Chemical Co. (St. Louis, MO). Amersham Biosciences (Arlington Heights, IL) supplied the $[4\text{-}^{14}\text{C}]\text{Ch}$ (~ 55 mCi/mmol in toluene), referred to as $[^{14}\text{C}]\text{Ch}$. Before incorporation into liposomes or cells, the $[^{14}\text{C}]\text{Ch}$ was separated from any preexisting oxidation products by normal-phase HPLC, as described (10). Avanti Polar Lipids (Alabaster, AL) supplied the 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC). HPLC-grade organic solvents were from Mallinkrodt (Paris, KY). The primary ChOOHs 5 α -OOH and 6 β -OOH were prepared by aluminum phthalocyanine disulfonate-sensitized photooxidation of Ch in pyridine, followed by reverse-phase HPLC separation of the two isomers (11). After confirmation of identity by NMR (12), a stock solution of each ChOOH in isopropanol was analyzed for peroxide concentration by iodometric assay (13) and stored at -20°C . We decided not to isolate and test 6 α -OOH in study because its photooxidation yield is considerably lower than that of 6 β -OOH (5). ^{14}C -labeled 7 α -OOH, 7 β -OOH, 7 α -OH, and 7 β -OH, used as standards for HPTLC-PI-based ChOX analyses, were prepared as described previously (11,14); 5,6-epoxide, also used as a standard, was obtained from Steraloids Inc. (Wilton, NH). The following stock solutions were used for inducing ChOOH one-electron reduction: 1.0 mM ferric-8-hydroxyquinoline ($\text{Fe}(\text{HQ})_3$) (15), and 100 mM ascorbic acid in Chelex-treated PBS, prepared immediately before an experiment.

Liposome preparation

Large (100 nm) unilamellar vesicles (LUVs) were fabricated by an extrusion process, using an apparatus from Lipex Biomembranes (Vancouver, BC) and 0.1 μm polycarbonate filters (5,10). The typical composition of a liposome preparation for assessing $\text{Fe}(\text{HQ})_3/\text{AH}^-$ -induced ChOOH reductive decay and $[^{14}\text{C}]\text{ChOX}$ accumulation was as follows: 1.0 mM DMPC or POPC, 0.75 mM (~ 0.5 $\mu\text{Ci/ml}$) $[^{14}\text{C}]\text{Ch}$, and 0.05 mM 5 α -OOH or 6 β -OOH in

bulk phase PBS. Before use the PBS was treated with Chelex-100 to deplete redox metal ions that might catalyze premature ChOOH loss (10). Other details were as described previously (5,10). The LUVs were stored under argon at 4 °C and used experimentally within 48 h.

Cell culture conditions

Murine L1210 leukemia cells, obtained from the American Type Culture Collection (Rockville, MD), were grown in RPMI 1640 medium supplemented with 10% serum, growth factors, and antibiotics, using standard culture conditions (14). Cells in serum-free medium were labeled with [^{14}C]Ch by spontaneous transfer from liposomes, using a published procedure (15). The [^{14}C]Ch delivered by this approach is located predominantly in the plasma membrane. All experiments were carried out on exponentially growing cells.

Measurement of ChOOH reductive loss

PC/[^{14}C]Ch/ChOOH (20:15:1 by mol) LUVs in PBS, where PC is DMPC or POPC and ChOOH is 5 α -OOH or 6 β -OOH, were incubated at 37 °C in the presence of 1.0 μM Fe(HQ) $_3$ and 1.0 mM AH $^-$ (added last). Controls without Fe(HQ) $_3$ or AH $^-$ were incubated alongside. At various time points, samples were removed, extracted with chloroform/methanol (2:1 by vol) and recovered lipid fractions were analyzed for remaining ChOOH by high-performance liquid chromatography with mercury cathode electrochemical detection [HPLC-EC(Hg)], using previously specified conditions (5,15).

Determination of ChOOH-induced chain lipid peroxidation

Lipid extracts from Fe(HQ) $_3$ /AH $^-$ -treated PC/[^{14}C]Ch/ChOOH LUVs or [^{14}C]Ch-labeled L1210 cells exposed to ChOOH-containing LUVs were analyzed for [^{14}C]ChOX species (7 α / β -OOH, 7 α / β -OH, 5,6-epoxide) by means of high performance thin layer chromatography with phosphorimaging radiodetection (HPTLC-PI). Well-separated Ch, the loss of which to ChOX was usually negligible, was used as an internal loading standard. Additional details about methodology and evaluation of [^{14}C]ChOX yields were as described previously (15).

Assessment of ChOOH cytotoxicity

L1210 cells ($\sim 7.5 \times 10^5/\text{ml}$ in 1% serum-containing RPMI medium) were treated with POPC/Ch/5 α -OOH or POPC/Ch/6 β -OOH LUVs in a 96-well tissue culture plate such that the starting concentration of ChOOH in bulk suspension was 50 μM . All mixtures were standardized to the same concentration of total LUV lipid to normalize any background toxicity due to lipid alone. After LUV addition, the cells were returned to the incubator for 24 h and then assessed for survival by clonal assay (14).

Results

Iron-mediated one-electron reduction of 5 α -OOH vs. 6 β -OOH in liposomal membranes

In initial experiments we compared liposomal 5 α -OOH and 6 β -OOH for susceptibility to one-electron reduction and ability to set off chain lipid peroxidation reactions. As shown in Fig. 1A, 5 α -OOH and 6 β -OOH, each at ~ 2.8 mol % in separate POPC/[^{14}C]Ch/ChOOH LUVs, were reduced at the same apparent first order rate ($k \sim 0.087 \text{ min}^{-1}$) when incubated with Fe(HQ) $_3$ and AH $^-$. No reaction occurred when iron was omitted (Fig. 1A), in agreement with earlier evidence that iron plays an essential catalytic role in shuttling an electron from AH $^-$ to the hydroperoxide (15). As shown previously for 5 α -OOH and 7 α -OOH (15), this one-electron step is rate-limiting in overall reduction of ChOOH to ChOH (diol), its rate increasing proportionately with iron concentration. When incubated with Fe(HQ) $_3$ /AH $^-$, 5 α -

OOH and 6 β -OOH in separate DMPC/[¹⁴C]ChOOH LUVs (where non-oxidizable DMPC was substituted for oxidizable POPC) once again decayed at the same rate ($k \sim 0.05 \text{ min}^{-1}$), as shown in Fig. 1B. The reason for the approximately 40% lower rate constant in the DMPC-containing vs. POPC-containing LUVs is not clear, but one possibility is that it reflects a fluidity difference which decreases access of AH⁻ to membrane-bound ferric iron or ChOOH to ferrous iron.

Chain peroxidation-initiating potency of 5 α -OOH vs. 6 β -OOH in liposomal membranes

In addition to tracking ChOOH decay in Fe(HQ)₃/AH⁻-treated PC/[¹⁴C]Ch/ChOOH liposomes, we tracked [¹⁴C]ChOX formation as a measure of ChOOH chain-initiating potency. As shown by the HPTLC-PI results in Fig. 2A, reductive activation of POPC/[¹⁴C]Ch/5 α -OOH LUVs resulted in progressive accumulation of [¹⁴C]ChOX species, including (in order of increasing mobility) 7 α -OH, 7 β -OH, 5,6-epoxide, 7 α -OOH, and 7 β -OOH. No significant formation of these species was observed in a control that contained AH⁻, but not Fe(HQ)₃ (results not shown), consistent with the non-decay of control 5 α -OOH seen in Fig. 1A. In striking contrast to 5 α -OOH, 6 β -OOH in POPC/[¹⁴C]Ch/6 β -OOH LUVs produced few, if any, net ChOX during incubation with Fe(HQ)₃/AH⁻ (Fig. 2A). The trace amount of 5,6-epoxide (band 3) was present from the outset and remained constant. The concentration of total ChOX in bulk LUV suspension increased progressively with reaction time in the 5 α -OOH system, reaching $\sim 25 \mu\text{M}$ by 30 min, but it remained at $< 1 \mu\text{M}$ throughout in the 6 β -OOH system (Fig. 2B).

We also examined ChOX buildup in Fe(HQ)₃/AH⁻-activated DMPC/[¹⁴C]Ch/ChOOH liposomes, where Ch was the only oxidizable lipid. As shown in Fig. 3, the concentration of total net ChOX in DMPC/[¹⁴C]Ch/5 α -OOH LUVs increased rapidly with incubation time out to about 10 min and then more slowly, reaching $\sim 22 \mu\text{M}$ by 30 min. However, the ChOX yield in DMPC/[¹⁴C]Ch/6 β -OOH LUVs was barely detectable throughout, i.e. $< 1 \mu\text{M}$. In both types of liposome, therefore, 5 α -OOH and 6 β -OOH underwent one-electron reduction at the same rate (Fig. 1 A, B), only 5 α -OOH was able to initiate free radical-mediated lipid peroxidation (Figs. 2 and 3). The fact that POPC is oxidizable, but DMPC is not, probably accounts the greater initial rate of 5 α -OOH-induced ChOX accumulation in the Fig. 3 experiment compared with the Fig. 2. In the latter case, POPC would have competed with [¹⁴C]Ch for a chain-initiating oxyl radical, whereas in the former case no phospholipid competition was possible, permitting faster early ChOX buildup. The later stage slowdown in 5 α -OOH-induced ChOX (Fig. 3) is attributed to increasing termination due to combination of Ch-derived radicals, events that would be less frequent in the POPC system (Fig. 2).

Toxic free radical lipid peroxidation in 5 α -OOH- vs. 6 β -OOH-treated L1210 cells

We asked whether the contrasting effects of 5 α -OOH and 6 β -OOH one-electron turnover in liposomal membranes would also be manifested at the cellular level. To examine this, we exposed [¹⁴C]Ch-labeled L1210 cells to POPC/Ch/5 α -OOH or POPC/Ch/6 β -OOH LUVs, using initial ChOOH concentrations of 20 μM and 50 μM , and assessed [¹⁴C]ChOX accumulation and cell survival after an incubation period of 24 h. As shown in Fig. 4, a substantial yield of total ChOX was generated by 5 α -OOH, 20 μM and 50 μM giving $\sim 6 \mu\text{mol}/10^6 \text{ cells}$ and $\sim 11 \mu\text{mol}/10^6 \text{ cells}$, respectively. In striking contrast, 6 β -OOH, even at the higher concentration, produced no more than $\sim 0.5 \mu\text{mol}/10^6 \text{ cells}$. Using a clonogenicity assay, we found that cell survival after 24 h exposure to 50 μM LUV ChOOH was as follows: $2 \pm 1\%$ for 5 α -OOH; $95 \pm 2\%$ for 6 β -OOH; means $\pm$ SE ($n=3$). Clearly, therefore, 5 α -OOH was much more cytotoxic than 6 β -OOH, and this correlated with 5 α -OOH's ability to induce vigorous chain peroxidation and 6 β -OOH's inability to do so.

Discussion

Most lipid hydroperoxides (LOOHs) in membrane environments, including 5 α -OOH and 7 α / β -OOH, are susceptible to iron-catalyzed one-electron reduction, and this can exacerbate membrane damage by triggering chain lipid peroxidation (3,4,15). In the case of 5 α -OOH, the effects of a non-radical primary reaction ($^1\text{O}_2$ addition to Ch) are intensified by secondary free radical reactions, whereas with 7 α / β -OOH, free radical reactions are propagated. We found unexpectedly in the present study that another $^1\text{O}_2$ adduct, 6 β -OOH, though reduced by $\text{Fe}(\text{HQ})_3/\text{AH}^-$ at the same rate as 5 α -OOH in liposomal membranes, lacked the latter's ability to set off damaging chain peroxidation cascades. In consistent fashion, liposomal 6 β -OOH was found to be minimally prooxidant and cytotoxic toward L1210 cells compared with liposomal 5 α -OOH. In an earlier study with oxidizable liposomes prepared with the same amount of either 5 α -OOH or 7 α -OOH, we showed that these ChOOHs decayed at the same initial rate during $\text{Fe}(\text{HQ})_3/\text{AH}^-$ treatment and that the rate of [^{14}C]ChOX accumulation was also the same, indicating that 5 α -OOH and 7 α -OOH have the same chain-initiating potency (15). Both of these rates were dependent on $\text{Fe}(\text{HQ})_3$ concentration, consistent with a two one-electron reduction steps, the first (rate-limiting) step being ChOOH reduction to an oxyl radical (ChO $\cdot$) by Fe^{2+} , and the second step being reduction of ChO $\cdot$ to the alcohol (ChOH) by an oxidizable lipid. Ascorbate reduces Fe^{3+} to Fe^{2+} for the first step and competes with a lipid reductant in the second step. In another study (5), we showed that preexisting 5 α -OOH, 6 α -OOH, and 6 β -OOH in photoperoxidized liposomes all decayed at the same rate during incubation with $\text{Fe}(\text{HQ})_3/\text{AH}^-$, suggesting equal first-step reducibility. Although [^{14}C]ChOX buildup was not determined in this work (5), 6 β -OOH was shown to be much less cytotoxic toward L1210 cells than 5 α -OOH; e.g. at 100 μM ChOOH, 6 β -OOH had an 8-fold lower $\log_{10}$ reduction value in a clonogenicity assay. In seeking an explanation for this, we compared the susceptibilities of 5 α -OOH, 6 β -OOH, and 7 α -OOH to GPx4-catalyzed reductive inactivation. (GPx4 remains the only enzyme known to be capable of detoxifying ChOOHs.) Kinetic experiments with both isolated and cell-borne enzyme revealed that 6 β -OOH was the most reactive GPx4 substrate and 5 α -OOH the least reactive, possibly due to its unique tertiary structure (14). This evidence suggested that 6 β -OOH's limited cytotoxicity compared with 5 α -OOH was simply due to its rapid reductive inactivation by GPx4. Although this could be a contributing factor, we now know, based on the present study, that 6 β -OOH lacks the strong prooxidant properties of 5 α -OOH and 7 α / β -OOH, and this could be the most important reason for it being relatively innocuous. We examined only the β -epimer of the 6-positional hydroperoxide in this study due to the relatively low yield of the α -epimer in the photopreparative procedure used (5,8,11).

How can one account for the insignificant chain-initiating potency and cytotoxicity of 6 β -OOH compared with 5 α -OOH? A reasonable deduction based on the data presented is that both hydroperoxides in $\text{Fe}(\text{HQ})_3/\text{AH}^-$ -treated liposomes were equally prone to initial one-electron reduction by ferrous iron, but differed in their susceptibility to the second one-electron reduction involving hydrogen abstraction from an oxidizable lipid molecule, i.e. the actual chain-initiating step. This difference might be explained on thermodynamic grounds, i.e. the redox potential of 6 β -O $\cdot$ might be significantly lower than that of 5 α -O $\cdot$ such that the former lacks lipid oxidizing ability. Alternatively, a kinetic argument might apply, i.e. 6 β -O $\cdot$ may abstract lipid hydrogens at a far lower rate than 5 α -O $\cdot$, possibly due to some structural factor, e.g. unusual orientation in the membrane bilayer. Distinguishing between these possibilities will require additional study; however, *viz.* determination of redox potentials for the two ChOOHs. At present, the kinetic explanation is tentatively more appealing because liposomal 6 β -OOH does appear to give rise to at least some ChOX after prolonged incubation with iron and ascorbate.

This study places additional emphasis on the importance of 5 α -OOH as a $^1\text{O}_2$ adduct with cytotoxic and redox signaling properties (3-5). In mammalian tissues under $^1\text{O}_2$ attack, e.g. long-wavelength UVA-exposed skin (16,17) or blue light-exposed retinal pigment epithelium (18), 5 α -OOH would be generated more rapidly than any other ChOOH isomer, but detoxified most slowly by the GSH/GPx4 system. Accordingly, one would expect 5 α -OOH to have a longer lifetime and accumulate to higher steady state levels than other ChOOHs and possibly also phospholipid hydroperoxides, given that the latter are much better GPx4 substrates than ChOOHs (19). Although 5 α -OOH is known to undergo allylic rearrangement to 7 α -OOH, this is typically insignificant at low levels of membrane peroxidation, as in a moderately $^1\text{O}_2$ -stressed cell (5). In addition to undergoing one- and two-electron turnover in a membrane of origin, LOOHs can desorb and transfer to other membranes, where such turnover may occur. If antioxidant capacity of acceptor compartments is insufficient, transfer will expand LOOH cytotoxic and redox signaling action (6). All ChOOHs translocate faster than phospholipid counterparts, but among the former, 5 α -OOH moves significantly faster than 6 β -OOH, both spontaneously and when assisted by a lipid transfer protein (6). Based on these established characteristics of 5 α -OOH (fastest $^1\text{O}_2$ -mediated generation, slowest enzymatic detoxification, relatively rapid intermembrane transfer) and our evidence herein that 6 β -OOH is incapable of launching membrane-damaging free radical peroxidation, 5 α -OOH stands out as the most dangerous $^1\text{O}_2$ adduct of Ch and as potentially more dangerous than $^1\text{O}_2$ adducts of natural phospholipids.

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Abbreviations

AH ⁻	ascorbate
AlPcS ₄	aluminum phthalocyanine tetrasulfonate
Ch	cholesterol
ChOOH(s)	cholesterol hydroperoxide(s)
ChOX	cholesterol oxidation product(s)
DMPC	dimyristoyl- <i>sn</i> -glycero-3-phosphocholine
Fe(HQ) ₃	ferric-8-hydroxyquinoline
GPx4	type-4 glutathione peroxidase
HPLC-EC(Hg)	high-performance liquid chromatography with mercury cathode electrochemical detection
HPTLC-PI	high-performance thin layer chromatography with phosphorimaging detection
LOOH(s)	lipid hydroperoxide(s)
LUV(s)	large unilamellar vesicles
PBS	Chelex-treated phosphate-buffered saline (25 mM sodium phosphate, 125 mM sodium chloride, pH 7.4)
PC	phosphatidylcholine
POPC	1-palmitoyl-2-oleoyl- <i>sn</i> -glycero-3-phosphocholine
5 α -OOH	3 β -hydroxy-5 α -cholest-6-ene-5-hydroperoxide
6 α - and 6 β -OOH	3 β -hydroxycholest-4-ene-6 α - and 6 β -hydroperoxide

7α - and 7β -OOH	3β -hydroxycholest-5-ene- 7α - and 7β -hydroperoxide
7α - and 7β -OH	cholest-5-ene- 3β , 7α - and 7β -diol

Figure 1.

Time courses for one-electron reduction of liposomal 5α-OOH and 6β-OOH. PC/[¹⁴C]Ch/ChOOH (20:15:1 by mol) LUVs in PBS were incubated at 37 °C in the presence of 1.0 μM Fe(HQ)₃ and 1.0 mM AH⁻. At the indicated times, samples were extracted and recovered lipid fractions were analyzed for remaining ChOOH by means of HPLC-EC(Hg). Panel A: PC was POPC and ChOOH was 5α-OOH (○) or 6β-OOH (Δ). Panel B: PC was DMPC and ChOOH was 5α-OOH (○) or 6β-OOH (Δ). In (A) and (B), control systems containing AH⁻, but lacking Fe(HQ)₃, were also analyzed for 5α-OOH (●) and 6β-OOH (▲). Data in (A) and (B) are from one of the experiments represented in Fig. 2 and Fig. 3, respectively.

Figure 2.

Comparison of 5α-OOH and 6β-OOH as chain peroxidation inducers in POPC-based liposomes. POPC/[¹⁴C]Ch/5α-OOH and POPC/[¹⁴C]Ch/6β-OOH (20:15:1 by mole) LUVs in PBS were individually incubated in the presence of 1.0 μM Fe(HQ)₃ and 1.0 mM AH⁻ at 37 °C. At the indicated time points out to 20 min, samples were removed, extracted, and recovered lipid fractions were analyzed by HPTLC-PI. (A) [¹⁴C]ChOX profiles over a 0-20 min reaction period for 5α-OOH- and 6β-OOH-containing LUVs. Total lipid per lane: 360 nmol. Notations: (O) origin; (1) 7α-OH; (2) 7β-OH; (3) 5,6-epoxide; (4) 7α-OOH; (5) 7β-OOH; (6) Ch; (F) solvent front. (B) Concentration of total accumulated ChOX (bands 1-5) in the reaction mixture as a function of incubation time: (○) 5α-OOH; (Δ) 6β-OOH. Plotted

data are means $\pm$ SE of values from three replicate experiments, one of which is represented in (A).

Figure 3.

Comparison of 5α-OOH and 6β-OOH as chain peroxidation inducers in DMPC-based liposomes. DMPC/[¹⁴C]Ch/5α-OOH and DMPC/[¹⁴C]Ch/6β-OOH (20:15:1 by mole) LUVs in PBS were individually incubated in the presence of 1.0 μM Fe(HQ)₃ and 1.0 mM AH⁻ at 37 °C. At various times, samples were extracted and recovered lipid fractions subjected to HPTLC-PI analysis (360 nmol lipid per lane). Plots show concentration of total ChOX in the reaction mixture as a function of incubation time: (○) 5α-OOH; (Δ) 6β-OOH. Data are means ± SE of values from three separate experiments.

Figure 4.

Chain-initiating potency of 5α-OOH vs. 6β-OOH in L1210 cells. [¹⁴C]Ch-labeled L1210 cells ($\sim 2 \times 10^6$ /ml in 1% serum-containing RPMI medium) were mixed with POPC/Ch/5α-OOH or POPC/Ch/6β-OOH (10:3:3 by mol) LUVs, giving 20 μM starting ChOOH and 0.11 mM total LUV lipid in bulk phase. Cells were also treated with 50 μM LUV-ChOOH (0.27 mM total lipid). POPC/Ch (10/6 by mol) LUVs were used in control mixtures. After 24 h of incubation at 37 °C, lipids were extracted from cell samples and analyzed for [¹⁴C]ChOX species by HPTLC-PI. Total ChOX levels for cells treated with ChOOHs, corrected for non-ChOOH controls, are plotted as a function of starting ChOOH concentration. Means $\pm$ SE of values from three replicate experiments are shown.