Inhibition of Lipid Oxidation

Philippe Boucher and Hans Gerhard Vogel

Contents

General Considerations 2285

Inhibition of Lipid Peroxidation of Isolated Plasma Low-Density Lipoproteins 2286
Purpose and Rationale 2286
Procedure 2286
Evaluation 2287
Modifications of the Method 2287

References and Further Reading 2288

General Considerations

Oxidative modification of the low-density lipoproteins (LDL) has been shown to cause accelerated degradation of LDL via the scavenger receptor pathway. Under conditions of high serum LDL levels, LDL particles can migrate into the subendothelial space where oxidation of LDL can occur (Heinecke 1998; Jiang et al. 2011). The actual oxidation process is believed to begin with lipid peroxidation, followed by fragmentation to result in short-chain aldehydes. These aldehydes can form adducts with the lysine residues of apo B, creating a new epitope which is recognized by the scavenger receptor of macrophages.

During the same process, lecithin is converted to lysolecithin, which is a selective chemotactic agent for monocytes. The monocytes adhere to the arterial wall and penetrate through to the subendothelium. Once there, the monocyte changes to a tissue macrophage which takes up the oxidized LDL via the scavenger receptor. The uptake of oxidized LDL continues until the macrophage is engorged with cholesteryl esters ultimately forming a foam cell. Groups of these foam cells constitute a fatty streak. By inhibiting the oxidation of LDL, it is hoped that the modification of apo B and the production of chemotactic lysolecithin can be prevented.

The family of receptors for mammalian low-density proteins has been reviewed by Hussain et al. (1999).

Hans Gerhard Vogel: deceased.

P. Boucher (✉)
Department of Physiology, Université de Strasbourg, UMR CNRS 7213, Illkirch Cedex, France
e-mail: philippe.boucher@unistra.fr; philippe_boucher@yahoo.com

H.G. Vogel
Aalen, Germany

© Springer International Publishing Switzerland 2016

F.J. Hock (ed.), *Drug Discovery and Evaluation: Pharmacological Assays*,
DOI 10.1007/978-3-319-05392-9_51

References and Further Reading

Bruckdorfer KR (1990) Free radicals, lipid peroxidation and atherosclerosis. Curr Opin Lipidol 1:529–535

Esterbauer H, Rotheneder M, Striegl G, Waeg G, Ashy A, Sattler W, Jürgens G (1989) Vitamin E and other lipophilic antioxidants protect LDL against oxidation. Fat Sci Technol 91:316–324

Hussain MM, Strickland DK, Bakillah A (1999) The mammalian low-density lipoprotein receptor family. Annu Rev Nutr 19:141–172

Jürgens G (1989) Modified serum lipoproteins and atherosclerosis. Ann Rep Med Chem 25:169–176

McCarthy PA (1993) New approaches to atherosclerosis: an overview. Med Res Rev 13:139–159

Parthasarathy S, Wieland E, Steinberg D (1989) A role for endothelial cell lipoxygenase in the oxidative modification of low density lipoprotein. Proc Natl Acad Sci U S A 86: 1046–1050

Rankin SM, Parthasarathy S, Steinberg D (1991) Evidence for a dominant role of lipoxygenase(s) in the oxidation of LDL by mouse peritoneal macrophages. J Lipid Res 32:449–456

Steinberg D (1990) Arterial metabolism of lipoproteins in relation to atherogenesis. In: Lee KT, Onodera K, Tanaka K (eds) Atherosclerosis II: recent progress in atherosclerosis research, vol 598, Annals of the New York Academy of Sciences. pp 188–193

Steinbrecher UP (1987) Oxidation of human low density lipoprotein results in derivatization of lysine residues of apolipoprotein B by lipid peroxide decomposition products. J Biol Chem 262:3603–3608

Steinbrecher UP (1990) Oxidatively modified lipoproteins. Curr Opin Lipidol 1:411–415

Steinbrecher UP, Witztum JL, Parthasarathy S, Steinberg D (1987) Decrease in reactive amino groups during oxidation or endothelial cell modification of LDL. Arteriosclerosis 7:135–143

Steinbrecher UP, Zhang H, Lougheed M (1990) Role of oxidatively modified LDL in atherosclerosis. Free Rad Biol Med 9:155–158

Witztum JL, Steinberg D (1991) Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 88:1785–1792

Inhibition of Lipid Peroxidation of Isolated Plasma Low-Density Lipoproteins

Purpose and Rationale

Hypercholesterolemic Watanabe rabbits are considered to be a suitable model to study the effect of antioxidants as antiatherosclerotic agents (Carew et al. 1987; Kita et al. 1987; Steinberg et al. 1988; Dresel et al. 1990). Plasma of Watanabe heritable hyperlipidemic (WHHL) rabbits is used to test the inhibition of Cu^{2+}-induced lipid peroxidation of isolated low-density lipoproteins (LDL).

Procedure

Animals of a modified Watanabe heritable hyperlipidemic rabbit strain (Gallagher et al. 1988) are used. The animals are fed over a period of 12 weeks with Purina rabbit chow diet with or without 1 % of test compound or standard (probucol). Plasma samples are collected in Na_2EDTA (0.1 % final concentration). LDLs are isolated from each rabbit plasma using a sequential ultracentrifugation technique at $d = 1.019–1.063$ g/ml (Mao et al. 1983). LDLs are then dialyzed against phosphate-buffered saline (PBS, 0.01 M sodium phosphate, 0.12 M NaCl, pH 7.4) at 4 °C for 24 h.

For determination of LDL lipid peroxidation induced by Cu^{2+}, 100 µg of each LDL sample is adjusted to a volume of 1.5 ml with distilled water. Lipid peroxidation is initiated by addition of $CuSO_4$ to a final concentration of 5 µM followed

by an incubation at 37 °C for 3 h. The reaction is stopped by adding 100 µl of 50 mM Na_2EDTA. Fifty micrograms of LDL from the reaction mixture are added to 1.5 ml of 20 % trichloroacetic acid and vortexed. Finally, 1.5 ml of 0.67 % thiobarbituric acid (TBA) in 0.05 N NaOH is added, and the mixture is incubated at 90 °C for 30 min. Samples are centrifuged at 1,500 rpm for 10 min. The absorbance of the supernatant fractions is determined at 532 nm to estimate the content of lipid peroxides (thiobarbituric acid-reactive substances). A standard curve (0–5 nmol) of malondialdehyde is generated using malondialdehyde bis(dimethyl acetal) as reference to determine the lipid peroxidation content in Cu^{2+}-treated LDL.

Evaluation

The content of lipid peroxide in LDL is plotted against the drug concentration in LDL fractions. The extent of Cu^{2+}-induced peroxidation decreases with increasing drug concentrations. The effects of test compounds are compared to the standard.

Modifications of the Method

Inhibition of iron-dependent lipid peroxidation by test compounds was measured by Braughler et al. (1987) and Yoshioka et al. 1989).

Yamamoto et al. (1986) studied the effects of probucol on lipid storage in macrophages in vitro in the presence of acetylated low-density lipoprotein using macrophage-like cells (UE-12) established from a human histiocytic lymphoma cell line.

Barnhart et al. (1989) used LDL from human plasma to study the concentration-dependent antioxidant activity of probucol.

Parthasarathy et al. (1986) incubated LDL from human plasma samples with rabbit aortic endothelial cells and measured the increase in electrophoretic mobility, the increase in peroxides, and the increase in subsequent susceptibility to macrophage degradation.

Mansuy et al. (1986) studied the inhibition of lipid peroxidation induced in liver microsomes either chemically by $FeSO_4$ and reducing agents (cysteine or ascorbate) or enzymatically by NADPH and CCl_4.

References and Further Reading

Asakawa T, Matsushita S (1980) Coloring conditions of thiobarbituric acid test for detecting lipid hydroperoxides. Lipids 15:137–140

Barnhart RL, Busch SJ, Jackson RL (1989) Concentration-dependent antioxidant activity of probucol in low density lipoproteins in vitro: probucol degradation precedes lipoprotein oxidation. J Lipid Res 30:1703–1710

Bernheim F, Bernheim MLC, Wilbur KM (1948) The reaction between thiobarbituric acid and the oxidation products of certain lipids. J Biol Chem 174:257–264

Braughler JM, Pregenzer JF, Chase RL, Duncan LA, Jacobsen EJ, McCall JM (1987) Novel 21-amino steroids as potent inhibitors of iron-dependent lipid peroxidation. J Biol Chem 262:10438–10440

Carew TE, Schwenke DC, Steinberg D (1987) Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. Proc Natl Acad Sci U S A 84:7725–7729

Dresel HA, Deigner HP, Frübis J, Strein K, Schettler G (1990) LDL-metabolism of the arterial wall – new implications for atherogenesis. Z Kardiol 79(Suppl 3):9–16

Gallagher PJ, Nanjee MN, Richards T, Roche WR, Miller NE (1988) Biochemical and pathological features of a modified strain of Watanabe heritable hyperlipidemic rabbits. Atherosclerosis 71:173–183

Heinecke JW (1998) Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis. Atherosclerosis 141:1–15

Jiang X, Yang Z, Chandrakala AN, Pressley D, Parthasarathy S (2011) Oxidized low density lipoproteins–do we know enough about them? Cardiovasc Drugs Ther 25:367–377

Kita T (1991) Oxidized lipoproteins and probucol. Curr Opin Lipidol 2:35–38

Kita T, Nagano Y, Yokode M, Ishii K, Kume N, Ooshima A, Yoshida H, Kawai C (1987) Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. Proc Natl Acad Sci U S A 84:5928–5931

Mansuy D, Sassi A, Dansette PM, Plat M (1986) A new potent inhibitor of lipid peroxidation in vitro and in vivo, the hepatoprotective drug anisyldithiolthione. Biochem Biophys Res Commun 135:1015–1021

Mao SJT, Patton JG, Badimon JJ, Kottke BA, Alley MC, Cardin AD (1983) Monoclonal antibodies to human plasma low-density lipoproteins. I. Enhanced binding of ^{125}I-labeled low-density lipoproteins by combined use of two monoclonal antibodies. Clin Chem 29:1890–1897

Mao SJT, Yates MT, Rechtin AN, Jackson RL, Van Sickle WA (1991) Antioxidant activity of probucol and its analogues in hypercholesterolemic Watanabe rabbits. J Med Chem 34:298–302

McLean LR, Hagaman KA (1989) Effect of probucol on the physical properties of low-density lipoproteins oxidized by copper. Biochemistry 28:321–327

Parthasarathy S, Young SG, Witztum JL, Pittman RC, Steinberg D (1986) Probucol inhibits oxidative modification of low density lipoprotein. J Clin Invest 77:641–644

Steinberg D, Parthasaraty S, Carew TE (1988) In vivo inhibition of foam cell development by probucol in Watanabe rabbits. Am J Cardiol 62:6B–12B

Yamamoto A, Takaishi S, Hara H, Nishikawa O, Yokoyama S, Yamamura T, Yamaguchi T (1986) Probucol prevents lipid storage in macrophages. Atherosclerosis 62:209–217

Yoshioka T, Fujita T, Kanai T, Aizawa Y, Kurumada T, Hasegawa K, Horikoshi H (1989) Studies with hindered phenols and analogues. 1. Hypolipidemic and hypoglycemic agents with ability to inhibit lipid peroxidation. J Med Chem 32:421–428

Zhang H, Basra HJK, Steinbrecher UP (1990) Effects of oxidatively modified LDL on cholesterol esterification in cultured macrophages. J Lipid Res 31:1361–1369

References and Further Reading

General Considerations

Bruckdorfer KR (1990) Free radicals, lipid peroxidation and atherosclerosis. Curr Opin Lipidol 1:529–535

Esterbauer H, Rotheneder M, Striegl G, Waeg G, Ashy A, Sattler W, Jürgens G (1989) Vitamin E and other lipophilic anti-oxidants protect LDL against oxidation. Fat Sci Technol 91:316–324

Hussain MM, Strickland DK, Bakillah A (1999) The mammalian low-density lipoprotein receptor family. Annu Rev Nutr 19:141–172

Jürgens G (1989) Modified serum lipoproteins and atherosclerosis. Ann Rep Med Chem 25:169–176

McCarthy PA (1993) New approaches to atherosclerosis: an overview. Med Res Rev 13:139–159

Parthasarathy S, Wieland E, Steinberg D (1989) A role for endothelial cell lipoxygenase in the oxidative modification of low density lipoprotein. Proc Natl Acad Sci U S A 86:1046–1050

Rankin SM, Parthasarathy S, Steinberg D (1991) Evidence for a dominant role of lipoxygenase(s) in the oxidation of LDL by mouse peritoneal macrophages. J Lipid Res 32:449–456

Steinberg D (1990) Arterial metabolism of lipoproteins in relation to atherogenesis. In: Lee KT, Onodera K, Tanaka K (eds) Atherosclerosis II: recent progress in atherosclerosis research, vol 598, Annals of the New York academy of sciences. pp 188–193

Steinbrecher UP (1987) Oxidation of human low density lipoprotein results in derivatization of lysine residues of apolipoprotein B by lipid peroxide decomposition products. J Biol Chem 262:3603–3608

Steinbrecher UP (1990) Oxidatively modified lipoproteins. Curr Opin Lipidol 1:411–415

Steinbrecher UP, Witztum JL, Parthasarathy S, Steinberg D (1987) Decrease in reactive amino groups during oxidation or endothelial cell modification of LDL. Arteriosclerosis 7:135–143

Steinbrecher UP, Zhang H, Lougheed M (1990) Role of oxidatively modified LDL in atherosclerosis. Free Rad Biol Med 9:155–158

Witztum JL, Steinberg D (1991) Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 88:1785–1792

Inhibition of Lipid Peroxidation of Isolated Plasma Low-Density Lipoproteins

Asakawa T, Matsushita S (1980) Coloring conditions of thiobarbituric acid test for detecting lipid hydroperoxides. Lipids 15:137–140

Barnhart RL, Busch SJ, Jackson RL (1989) Concentration-dependent antioxidant activity of probucol in low density lipoproteins in vitro: probucol degradation precedes lipoprotein oxidation. J Lipid Res 30:1703–1710

Bernheim F, Bernheim MLC, Wilbur KM (1948) The reaction between thiobarbituric acid and the oxidation products of certain lipids. J Biol Chem 174:257–264

Braughler JM, Pregenzer JF, Chase RL, Duncan LA, Jacobsen EJ, McCall JM (1987) Novel 21-amino steroids as potent inhibitors of iron-dependent lipid peroxidation. J Biol Chem 262:10438–10440

Carew TE, Schwenke DC, Steinberg D (1987) Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. Proc Natl Acad Sci U S A 84:7725–7729

Dresel HA, Deigner HP, Frübis J, Strein K, Schettler G (1990) LDL-metabolism of the arterial wall – new implications for atherogenesis. Z Kardiol 79(Suppl 3):9–16

Gallagher PJ, Nanjee MN, Richards T, Roche WR, Miller NE (1988) Biochemical and pathological features of a modified strain of Watanabe heritable hyperlipidemic rabbits. Atherosclerosis 71:173–183

Heinecke JW (1998) Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis. Atherosclerosis 141:1–15

Jiang X, Yang Z, Chandrakala AN, Pressley D, Parthasarathy S (2011) Oxidized low density lipoproteins–do we know enough about them? Cardiovasc Drugs Ther 25:367–377

Kita T (1991) Oxidized lipoproteins and probucol. Curr Opin Lipidol 2:35–38

Kita T, Nagano Y, Yokode M, Ishii K, Kume N, Ooshima A, Yoshida H, Kawai C (1987) Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. Proc Natl Acad Sci U S A 84:5928–5931

Mansuy D, Sassi A, Dansette PM, Plat M (1986) A new potent inhibitor of lipid peroxidation in vitro and in vivo, the hepatoprotective drug anisyldithiolthione. Biochem Biophys Res Commun 135:1015–1021

Mao SJT, Patton JG, Badimon JJ, Kottke BA, Alley MC, Cardin AD (1983) Monoclonal antibodies to human plasma low-density lipoproteins. I. Enhanced binding of ^{125}I-labeled low-density lipoproteins by combined use of two monoclonal antibodies. Clin Chem 29:1890–1897

Mao SJT, Yates MT, Rechtin AN, Jackson RL, Van Sickle WA (1991) Antioxidant activity of probucol and its analogues in hypercholesterolemic Watanabe rabbits. J Med Chem 34:298–302

McLean LR, Hagaman KA (1989) Effect of probucol on the physical properties of low-density lipoproteins oxidized by copper. Biochemistry 28:321–327

Parthasarathy S, Young SG, Witztum JL, Pittman RC, Steinberg D (1986) Probucol inhibits oxidative modification of low density lipoprotein. J Clin Invest 77:641–644

Steinberg D, Parthasaraty S, Carew TE (1988) In vivo inhibition of foam cell development by probucol in Watanabe rabbits. Am J Cardiol 62:6B–12B

Yamamoto A, Takaishi S, Hara H, Nishikawa O, Yokoyama S, Yamamura T, Yamaguchi T (1986) Probucol prevents lipid storage in macrophages. Atherosclerosis 62:209–217

Yoshioka T, Fujita T, Kanai T, Aizawa Y, Kurumada T, Hasegawa K, Horikoshi H (1989) Studies with hindered phenols and analogues. 1. Hypolipidemic and hypoglycemic agents with ability to inhibit lipid peroxidation. J Med Chem 32:421–428

Zhang H, Basra HJK, Steinbrecher UP (1990) Effects of oxidatively modified LDL on cholesterol esterification in cultured macrophages. J Lipid Res 31:1361–1369