Potent Antioxidant Properties of 4-Hydroxyl-propranolol

I. Tong Mak and William B. Weglicki

Department of Physiology and Experimental Medicine, Antioxidant Pharmacology Laboratory, George Washington University Medical Center, Washington, DC

Received August 1, 2003; accepted September 23, 2003

ABSTRACT

The antioxidant properties of 4-HO-propranolol (4HOP), a major metabolite of propranolol, were studied and compared with that of propranolol and vitamin E (Trolox). When isolated hepatic microsomal membranes were peroxidized by an iron-catalyzed \(^{•} \text{OH}\)-generating system \([\text{dihydroxyfumarate} + \text{Fe (III)}]\), 4HOP potently and concentration-dependently inhibited lipid peroxidation; the IC\(_{50}\) value was 1.1 \(\mu\text{M}\), whereas those for Trolox and propranolol were 4.3 and 168 \(\mu\text{M}\), respectively. When isolated human low-density lipoprotein (LDL) was oxidized by 7.5 \(\mu\text{M Cu(II)}\) for 9 h, 4HOP at 3 \(\mu\text{M}\) delayed the lag phase significantly by 108 min, which was comparable with that of probucol (98-min delay) but was far greater than that provided by propranolol (6 min) or Trolox (47 min). At 1 \(\mu\text{M}\) 4HOP, the delay was 45 min. When confluent cultured bovine aortic endothelial cells were exposed to the Fe-catalyzed oxy-radical system, acute loss of glutathione occurred (55% decrease in 50 min). Pretreatment of the cells with 0.067 to 6.7 \(\mu\text{M}\) 4HOP for 30 min provided increasing degrees of protection against the glutathione loss; the EC\(_{50}\) value was 1.2 \(\mu\text{M}\), whereas those for Trolox and propranolol were 7.9 and 49 \(\mu\text{M}\), respectively. The loss of cell survival due to the radical stress was also effectively preserved by 4HOP. In separate experiments, when the endothelial glutathione was oxidatively depleted by a peroxynitrite-generating system (3-morpholinosydnonimine), 4HOP also provided potent protective activities. In conclusion, 4HOP is 4- to 8-fold more potent than vitamin E and >100-fold more active than propranolol as a “chain-breaking” antiperoxidatant against membrane and LDL oxidation and can provide superior endothelial cytoprotective efficacy against oxygen- or nitrogen-derived oxidant-mediated cell injury. Being a major metabolite in human and with its plasma level approaching that of propranolol, 4-HO-propranolol may contribute, in part, to the cardiovascular therapeutic benefits of propranolol.

It has been well established that \(\beta\)-adrenoceptor blocking agents (\(\beta\)-blockers) have several beneficial cardiovascular effects in patients with hypertension, angina pectoris, myocardial infarction, and congestive heart failure (for review, see Nies, 1990). Propranolol was one of the first clinically used \(\beta\)-blockers, and it has been approved for more therapeutic indications than any other drug of its class (Nies, 1990). It was well recognized by the BHA study that chronic use of propranolol improved survival at relatively high and multiple daily doses (BHA Research Group, 1983); it also reduced the reinfarction rate in myocardial infarction patients and above all, reduced sudden cardiac death (BHA Research Group, 1983; Hjalmarson, 1997). The precise mechanisms for these effects remain unclear, but it became apparent in other trials that the latter property of propranolol was not shared by the more water soluble \(\beta\)-blockers such as atenolol and sotalol (Hjalmarson, 1997; Hjalmarson, 2000). The cardioprotective effect of propranolol was established in an animal model in which chronic treatment of propranolol was found able to provide protection against ischemia reperfusion injury (Khaper et al., 1997). In this study, tissue lipid peroxidation products, both before and after the ischemia-reperfusion episodes, were significantly reduced in the propranolol-treated hearts compared with control (Khaper et al., 1997). In an earlier study, Chobanian et al. (1985) observed that both D and L forms of propranolol were able to protect against atherosclerosis in cholesterol-fed rabbits, suggesting other nonpharmacological properties of propranolol might be beneficial. Because increased free radical generation was implicated in the pathogenesis of a variety of cardiovascular diseases, including ischemia/reperfusion injury, restenosis, and atherosclerosis (Steinberg et al., 1989; Halliwell and Gutteridge, 1990), we examined the membrane antiperoxidative activities of several \(\beta\)-blockers. We found that a number of

ABBREVIATIONS: BHA, Beta-Blocker Heart Attack Trial; 4HOP, 4-hydroxy-propranolol; LDL, low-density lipoprotein; PBS, phosphate-buffered saline; DHF, dihydroxyfumarate; TBARS, thioarbituric acid reactive substance; SIN-1, 3-morpholinosydnonimine; GSH, reduced glutathione; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ANOVA, analysis of variance; MDA, malondialdehyde; NO, nitric oxide; NLA, \(\alpha\)-naphthoxyacetic acid.
β-blockers exhibited concentration-dependent membrane antiperoxidative activity (Mak and Weglicki, 1988). Of the five β-blockers examined (propranolol, pindolol, metoprolol, atenolol, and sotalol), propranolol was the most potent agent (Mak and Weglicki, 1988), and the activities seemed to correlate with the drugs' hydrophobicity. Further studies indicated that the antioxidant activity of propranolol was independent of its pharmacological activity and was related to its intrinsic chemical properties rather than to its quinidine-like membrane stabilization effect (Mak and Weglicki, 1988; Mak et al., 1989; Weglicki et al., 1990). After our original report in 1988 (Mak and Weglicki, 1988), several other laboratories have confirmed the antioxidant properties of propranolol using membrane and cellular models (Janero et al., 1989; Aruoma et al., 1991; Anderson et al., 1996). However, the effective concentrations used in most of these studies (including our observations) are orders of magnitude (50–200 μM) higher than the reported serum clinical levels. Therefore, it is felt that the nonmetabolized propranolol in the serum even at the peak levels (~1 μM) may not be sufficient high enough to provide significant antioxidant activity. Although, due to its lipophilicity, higher levels of propranolol accumulated within membranes remain possible (Mason et al., 1991).

Propranolol is extensively metabolized (>90%) in vivo with less than 2% of the dose excreted unchanged in the urine (Walle et al., 1985; Nies, 1990). One metabolite is 4-HOH-propranolol (4-HOP); in this molecule, a hydroxyl group is added at the 4-position of the naphthalene moiety of propranolol (Fig. 1). This relatively stable metabolite has been found in substantial quantities in plasma after oral dosing (von Bahr et al., 1992; Walle et al., 1985; Nies, 1990). The present study was undertaken to characterize the antioxidant properties and potency of 4HOP by using a number of models: 1) Fe-superoxide-initiated lipid peroxidation in hepatic microsomes, 2) Cu²⁺-mediated LDL oxidation, and 3) oxy-radical- or peroxynitrite-mediated cytotoxicity in cultured endothelial cells.

Materials and Methods

Chemicals and Cell Culture. The propranolol metabolite 4-HO-propranolol (as hydrochloride) was a gift from Professor T. Walle (Medical University of South Carolina, Charleston, SC) and its purity was >97% (Oatis et al., 1981). LDL aliquots (L-2139) from human plasma, as well as most other chemicals were obtained from Sigma-Aldrich (St. Louis, MO). Sephadex G-25 M (PD-10) columns were purchased from Amersham Biosciences AB (Uppsala, Sweden). Bovine aortic endothelial cells (AG 07684) were obtained from National Institute on Aging, Aging Cell Culture Repository (Coriell Institute for Medical Research, Camden, NJ). The cells were cultured in Dulbecco’s minimal essential medium supplemented with 15% calf serum; the cells used in the experiments were between passages 8 to 13 as described previously (Mak et al., 1995).

Membrane Lipid Peroxidation. Hepatic microsomal membranes from male Sprague-Dawley rats were isolated essentially by differential centrifugation (Mak et al., 1998). The membranes (0.2 mg/ml) in PBS were preincubated with the propranolol analog for 20 min at 30°C before the addition of the free radical components, which consisted of 25 μM FeCl₃-250 μM ADP and 0.83 mM dihydroxyfumarate (DHF). After 15 min of incubation, the extent of lipid peroxidation was assessed by the TBARS method (Mak et al., 1986, 1998).

LDL Oxidation and Lag Time Determination. The EDTA content of the LDL samples was removed completely by gel filtration with PD-10 Sephadex G25-M filtration columns; PBS was used as the eluent (Mak et al., 1998). The LDL samples (50 μg of protein/ml PBS) were preincubated with or without (controls) (each drug 0.07–6.7 μM) for 30 to 45 min at 37°C. Oxidation of LDL was induced by final addition of 7.5 μM CuSO₄; the time course of LDL oxidation was followed up to 9 h and was measured by the increase of TBARS formation as described previously (Mak et al., 1998). Selected samples were incubated up to 24 h. The lag time before the onset of upward oxidation was estimated according to the method of Esterbauer et al. (1992) as described previously (Mak et al., 1998).

Endothelial Glutathione Loss and Cell Survival. Endothelial cells (>95% confluent in six-well plates) in a serum free Dulbecco’s phosphate-balanced salt buffer containing 10 mM glucose, pH 7.2, were preincubated with or without 0.2 to 6.7 μM of 4HOP for 30 min before the final addition of Fe (50 μM)-DHF (1.7 mM) or SIN-1 (0.25 mM) at 37°C (Dickens et al., 2002; Mak et al. 2002); the incubation period was normally for 50 min. Total cellular glutathione (>98% as GSH) was determined by the “cyclic method” using glutathione reductase (Mak and Weglicki, 1994; Mak et al. 2002). The effect of the drugs on oxy-radical- or SIN-1-impaired endothelial cell survival/growth was determined by the colorimetric MTT (tetrazolium) assay according to the procedure described previously (Mak et al., 1995; Dickens et al., 2002; Mak et al. 2002). Briefly, endothelial cells in 24-well flat-bottomed plates were washed two times with the balanced salt buffer containing 10 mM glucose and preincubated with the drugs in the same buffer for 30 min, 37°C. After drug treatment, the attached cells were incubated with either Fe-DHF or SIN-1 for 50 min. At the end of oxidant stress period, all wells were replaced with fresh normal growth medium and returned to the incubation chamber. Twenty-four hours later, all samples were quantified for viable cells by the MTT assay using the test wavelength of 570 nm and a reference one of 700 nm (Mak et al., 1995; Dickens et al., 2002; Mak et al. 2002). In all incubations, drug was dissolved initially in ethanol and subsequently diluted in the balanced salt buffer. The vehicle controls contained 0.25% (v/v) ethanol, reflecting identical level of ethanol in all samples with or without drug treatment. In separate samples, it was determined that vehicle alone produced less than 6% effect (N.S.) compared with the corresponding samples with no ethanol.

Statistical Analysis. All experiments were performed at least four times, and data were presented as means ± S.D. Statistical comparisons were performed by Student’s t test when only two treatment groups were compared; a value of p < 0.05 is considered statistically significant. Selected data were analyzed by one-way ANOVA followed by either a Dunnett’s multiple comparison or a Tukey’s test (Sigma Stat for Windows, version 2.03, 1997). The IC₅₀

Fig. 1. Chemical structures of propranolol (A) and 4-hydroxyl-propranolol (4HOP) (B).
or EC$_{50}$ values were calculated based on least square analysis from at least four drug concentrations.

**Results**

**Membrane Antioxidant Potency.** As an initial system to determine the antioxidant activity of 4HOP compared with propranolol and vitamin E, a microsomal membrane system was used, and lipid peroxidation was induced by our well established chemical superoxide and Fe-dependent, ‘OH-generating system (DHF + Fe) (Mak et al. 1986; Mak and Weglicki, 1994). The antioxidant activities of the drugs were measured as percentage of inhibition of the TBARS formation compared with the free radical controls, which were measured to be 56 ± 5 nmol (mean ± S.D., n = 6) of MDA equivalents/mg protein in 20 min of incubation. As represented by Fig. 2, all three agents provided concentration-dependent inhibition of the lipid peroxidation. Based on the log-concentration response curves, the IC$_{50}$ values were determined to be 1.1, 4.3, and 165 µM, respectively, for 4HOP, Trolox (a vitamin E analog), and propranolol. From a previous study using an identical membrane peroxidation system, we found that the IC$_{50}$ value for carvedilol, a well known multiple-action β-blocker with antioxidant activity, was 3.9 µM (Mak and Weglicki, 1995). Thus, it seems that 4HOP is 150-fold more potent than propranolol, and about 4-fold more potent than either Trolox or carvedilol as a membrane antioxidant. In separate control experiments, 4HOP (as well as propranolol and Trolox) up to 100 µM displayed no effect on the rate of DHF autoxidation and superoxide generation; none of these agents exhibited any detectable interference with the TBARS assay.

As an index of lipid peroxidation, we chose to use the TBARS method because of its convenience and cost-effectiveness. However, one must be aware of the limitation and specificity problems of this method. The TBARS assay may serve at best as a qualitative indication of lipid peroxidation in body fluids and tissue samples partly due to the presence of interfering compounds such as sugars, free amino acids, and bilirubin, and partly due to peroxide/MDA metabolic activities (Moore and Roberts, 1998; Halliwell and Gutteridge, 1999). However, in the absence of most of these interfering compounds and metabolic activities, the TBARS test can work reasonably well when applied to in vitro defined (and close) systems such as isolated microsomes and lipoproteins in buffers (Halliwell and Gutteridge, 1999). In these systems, the TBARS index may reflect mostly the extent of lipid peroxyl radical/lipid peroxide formation rather than the "original MDA level" (Halliwell and Gutteridge, 1999). Because lipid peroxyl radical/lipid peroxides are early stage active intermediates of the chain reactions, the assay can still be used as a semiquantitative index of lipid peroxidation in these in vitro systems.

**Inhibition against LDL Oxidation.** Because LDL oxidation has been implicated as an important contributing factor in the development of atherosclerosis (Steinberg et al., 1989), the following experiments were designed to assess the antioxidant potency of 4HOP against Cu$^{2+}$-mediated LDL oxidation (Mak et al., 1998). Under our conditions, upon addition of copper (7.5 µM), LDL oxidation at 37°C, determined by TBARS assay, followed a typical sigmoidal curve kinetic with an initial lag phase followed by a sharp propagation and final plateau phases (Fig. 3A). The extent of the lag time was interpreted as the antioxidant resistant capacity of the lipoprotein (Esterbauer et al., 1992). The lag time for the Cu$^{2+}$ controls was 57 ± 7 min (n = 5 ± S.D.). Figure 3A depicts the representative effects of the selected agents (at 3 µM) on the lag phase prolongation. Although propranolol had minimal and insignificant effect on the lag time delay (by 6 min), 4HOP delayed the lag time substantially and significantly longer (108 ± 16 min, p < 0.01; Fig. 2B). For comparison, Trolox provided intermediate effect (47 ± 10 min), whereas probucol, a well known anti-LDL-oxidation agent provided a significant delay (98 ± 13 min), which is not statistically different from that of 4HOP. Under similar condition, carvedilol at 3 µM provided a delay of 52 ± 6 min (p < 0.01 versus control). Figure 3B also indicates that significant and comparable effects were provided by 4HOP (45 min) and probucol (44 min) at 1 µM. In separate experiments, 4HOP at 0.3 µM delayed the lag phase by 21 ± 4 min (p < 0.05), and at 7 µM, could delay the lag phase up to 3 h, and at 20 µM, over 24 h.

**Endothelial Protective Effect against Losses of Glutathione and Cell Viability Mediated by Oxy-Radicals.** We assessed the cytotoxic protective capacity of 4HOP by using cultured endothelial cells subjected to the Fe-catalyzed ‘OH-producing free radical system. We have previously shown that the loss of endothelial glutathione is a sensitive indicator of acute oxidative stress (Mak et al., 1992; Mak and Weglicki, 1994). The ability of 4HOP to prevent the induced loss of glutathione was determined. As represented by Fig. 4A, the samples exposed to the oxy-radical-generating system in the absence of drug resulted in 55% loss of total glutathione (>95% as GSH) in 50 min. When the cells were pretreated with 4HOP before the addition of the free radical system, the loss of glutathione was concentration dependently attenuated with an EC$_{50}$ value of 1.2 µM. Significant protection was achieved by 4HOP at concentrations ≥0.2 µM (p < 0.05). For comparison, Trolox and propranolol also pro-

![Fig. 2. Potent inhibitory effect of 4HOP against microsomal lipid peroxidation induced by Fe-ADP + DHF. Hepatic microsomes (0.20 mg protein/ml) were preincubated with each agent for 20 min at 30°C before the addition of Fe-ADP and DHF. After 15 min of incubation, samples were assayed for TBARS formation and expressed as percentage of inhibition relative to the free radical controls. Values are means ± S.D. of five to six different determinations. The IC$_{50}$ values are calculated from the linear slopes of the graphs. Calculation for propranolol was based on data (not shown) derived from additional concentration range of 20 to 200 µM.](image-url)
mediated loss of GSH was examined. As for \( 3 \)H9262, 3H9262 with controls. Pretreatment of the cells with 0.2 to 2.0 \( \mu \)M propranolol, Trolox, probucol, and 4HOP to increase the resistance (lag time) of LDL against Cu\(^{2+}\)-induced peroxidation. Human LDL (50 \( \mu \)g of protein/ml) in PBS was preincubated with each agent (1 or 3 \( \mu \)M) for 30 min (37°C) before the addition of freshly prepared CuSO\(_4\) (7.8 \( \mu \)M). LDL lipid peroxidation was measured by increases in TBARS formation (absorbance at 532 nm). The lag time before the onset of upward oxidation was determined according to the method of Esterbauer et al. (1992). Values are derived from four to six different experimental determinations (mean ± S.D.). Lag time for the Cu(I) controls = 57 ± 7 min; \( t < 0.05 \) versus Cu(I) controls (performed by one-way ANOVA using Dunnett’s multiple comparison); \( t < 0.01 \) versus the corresponding propranolol groups (performed by one-way ANOVA using Tukey’s multiple test).

![Image](44x419 to 296x737)

Fig. 3. A, representative time course of Cu(II)-induced LDL lipid peroxidation with or without various drug treatment (3 \( \mu \)M) B, relative efficacy of 1 or 3 \( \mu \)M propranolol, Trolox, probucol, and 4HOP to increase the resistance (lag time) of LDL against Cu\(^{2+}\)-induced peroxidation. Human LDL (50 \( \mu \)g of protein/ml) in PBS was preincubated with each agent (1 or 3 \( \mu \)M) for 30 min (37°C) before the addition of freshly prepared CuSO\(_4\) (7.8 \( \mu \)M). LDL lipid peroxidation was measured by increases in TBARS formation (absorbance at 532 nm). The lag time before the onset of upward oxidation was determined according to the method of Esterbauer et al. (1992). Values are derived from four to six different experimental determinations (mean ± S.D.). Lag time for the Cu(I) controls = 57 ± 7 min; \( t < 0.05 \) versus Cu(I) controls (performed by one-way ANOVA using Dunnett’s multiple comparison); \( t < 0.01 \) versus the corresponding propranolol groups (performed by one-way ANOVA using Tukey’s multiple test).

![Image](326x462 to 542x737)

Fig. 4. Protective effects of 4HOP and Trolox against oxy-radical-induced loss of endothelial glutathione (A) and loss of cell survival (B). Confluent endothelial cells in six-well or 24-well plates were preincubated with 4HOP, Trolox, or propranolol for 30 min before the addition of the oxy-radical components (Fe/DHF). After 50 min of incubation, the samples from the six-well plates were processed for glutathione determination; the samples from the 24-well plates were replaced with fresh growth medium, and cell survival was determined by the MTT assay 24 h later. Total glutathione (>98% as GSH) for the 100% buffer control = 5.36 ± 0.8 nmol/10^6 cells. Values are means ± S.D. of four to six separate experiments; \( t < 0.001 \) versus vehicle controls [0.25% (v/v) ethanol] with no radicauls, \( t < 0.01 \) versus free radical (vehicle alone) alone (R). Vehicle alone (0.25% ethanol) had <6% (N.S.) effects on either the GSH or MTT results compared with the corresponding buffer controls.

Protection against SIN-1-Mediated Injury. We recently showed that confluence viability of red blood cells and endothelial cells were exposed to NO and superoxide anions simultaneously generated from SIN-1, dramatic depletion of cell GSH could occur due to the NO/O\(_2\)-derived peroxynitrite formation (Mak et al., 1996, 2002). In the following study, the effect of 4HOP on the NO/O\(_2\)-mediated loss of GSH was examined. As represented by Fig. 5, the sample exposed to SIN-1 at a dose of 0.25 mM for 50 min experienced a 56% loss of total glutathione and a correspondent 48% loss of viability determined 24 h later. Pretreatment of the with 4HOP (for 30 min) before the addition of SIN-1 provided dose-dependent protection against the loss of GSH; at 2 \( \mu \)M, 4HOP significantly attenuated the loss of glutathione (by 65%) and preserved the cell survival (back to 82% of control; \( t < 0.01 \)). At 2 \( \mu \)M, Trolox provided intermediate protective effects (~33% effect), but propranolol was ineffective (~10% effect). In data not shown, we observed similarly potent protective effects of 4HOP against peroxynitrite (25 \( \mu \)M)-mediated endothelial losses of glutathione and viability.

Discussion

The pharmacological and physiochemical properties of propranolol have been extensively studied (Nies, 1990; Mason et al., 1991); however, much less is known about the propranolol metabolites and their individual contributions to propranolol therapy. As mentioned above, we have reported that propranolol exhibits modest membrane antioxidant activity but...
only at a relatively high micromolar (20–200 μM) concentration (Mak and Weglicki, 1988; Mak et al., 1989). Compared with vitamin E, propranolol is at least 10-fold less effective as a membrane antioxidant. In the present study, we used three well defined in vitro models to compare the differential antioxidant potency between propranolol and 4HOP. With the membrane lipid peroxidation model, we demonstrated that the antiperoxidative potency of 4HOP is >150-fold more potent than its parent compound (Fig. 2). With the in vitro LDL study, 4HOP was more effective than propranolol and vitamin E in prolonging the lag phase of LDL oxidation. With the cultured endothelial cell model, based on acute loss of glutathione either mediated by an iron-dependent HO•-generating system or by a peroxynitrite-generating system, it was estimated that 4HOP was at least 40-fold more effective than propranolol as an endothelial cytoprotectant. More importantly, 4HOP displayed activity >5-fold more potent than vitamin E (or carvedilol). From a structural point of view, it is apparent that the presence of the HO group at the 4' position on the naphthalene moiety transforms the molecule into a vitamin E-like moiety with a phenol group attached to an aromatic resonance ring structure (naphthalene rings); the HO group probably confers the potent antioxidant activity in a manner similar to the phenol group of vitamin E (Burton and Ingold, 1981). With the HO group attached, 4HOP is more hydrophilic than its parent compound (Oatis et al., 1981), but it still retains a considerable degree of lipophilicity (Octanol/buffer partition coefficient ~3, compared with 18 for propranolol), which allows for its partitioning into cell membrane and LDL lipids. The observation (Fitzgerald and O'Donnell, 1971) that 4HOP provided a significant level of membrane stabilization activity in cardiac tissue supports this argument. Presumably, HOP may mediate its antioxidant action by a "chain-breaking" action within the membrane or LDL lipids similar to that mediated by vitamin E (Burton and Ingold, 1981). It is speculated that the higher activity of 4HOP is attributed to its lower redox potential comparing to vitamin E, perhaps due to the extra unsaturated ring structure. In our cultured cell model, we previously indicated that the loss of glutathione was due to its oxidative depletion by lipid peroxides (Mak et al., 1992, 2002); therefore, it is believed that the drug (4HOP)-mediated prevention of the glutathione loss was secondary to its chain-breaking action at the membrane level.

One important issue remains: Is the antioxidant potency of 4HOP pharmacologically relevant? One may approach this question by following the metabolic fate of propranolol. Once administered orally, propranolol is biotransformed rapidly in the liver into a number of metabolites before entering the systemic circulation (von Bahr et al., 1982; Walle et al., 1985). Of all the metabolites, 4HOP, α-naphthoylglucuronide (NLA), and propranolol glucuronide, which represent products of ring oxidation, side chain oxidation, and glucuronidation, respectively, are detected in substantial amounts in the blood (von Bahr et al., 1982; Walle et al., 1985). There are several other monophenolic isomers, i.e., 2,5,7’ OH-propranolols, which together could account for 33% of all monohydroxy propranolols in the rat (Bargar et al., 1983). However, in human, 4HOP accounts >85% of all mono-HO-propranolols (Walle et al., 1982). Once formed, 4HOP will be further metabolized mainly by glucuronic acid conjugation and then eliminated by the kidney (Walle et al., 1980, 1985; von Bahr et al., 1982). However, 4HOP may also be formed through deconjugation of HOP-glucuronide in tissues and blood due to enterohepatic recirculation (Walle et al., 1980, 1985; Bargar et al., 1983). On an average, the half-life of 4HOP is measured to be about 4 h, which is very close to that of propranolol (Walle et al., 1980, 1982). More importantly, plasma 4HOP levels can be as highs as that of propranolol, because, in general, they bear a one-to-one relationship, depending on the initial propranolol intake dose (Walle et al., 1982, 1989; Bargar et al., 1983). In one study (Walle et al., 1980), it was described that single oral dose of propranolol of 80 mg within 2 h after administration resulted in peak plasma 4HOP concentration of 0.2 μM. At lower doses (<40 mg), the peak 4HOP/propranolol ratio may approach 2 due to the fact that ring oxidation is the preferred initial step of propranolol metabolism (Walle et al., 1980). When the dose of propranolol was increased to 1 g/day, although the 4HOP/propranolol ratio fell below 1, it was estimated that the 4HOP level could approach 1 μM (Fitzgerald and O'Donnell, 1971; Walle et al., 1980). Because the present study demonstrated that significant protection against lipid peroxidation and endothelial oxidative injury could be achieved by submicromolar concentrations of 4HOP (Figs. 2–5), it is feasible that the antioxidant potency of 4HOP may be pharmacologically relevant especially in patients receiving high doses of propranolol. We speculate that the anti-LDL oxidative effect of 4HOP may explain the protective effect of propranolol (both β or τ forms) administration against the induced atherosclerosis in the rabbits observed by Chobanian et al. (1985). This property may also provide a plausible explanation for the reduced susceptibility of LDL to in vitro Cu²⁺-mediated oxidation in those patients with coronary disease on β-blocker therapy (Croft et al., 1992; Dimmitt et al., 1998).