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Rofecoxib Increases Susceptibility of Human LDL and Membrane Lipids to Oxidative Damage: A Mechanism of Cardiotoxicity [Original Articles]

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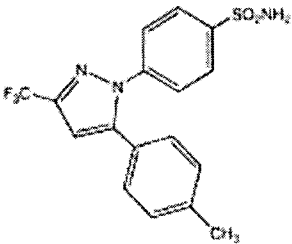
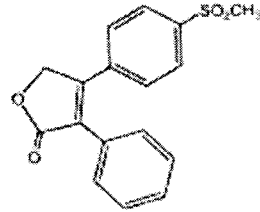
Abbreviations: COX, cyclooxygenase; NSAID, nonsteroidal anti-
inflammatory drug; LDL, low-density lipoprotein; MDA, malondialdehyde;
TBARS, thiobarbituric-acid-reactive-substances; DAPC, 1,2-diarachidonoyl-
sn-glycero-3-phosphocholine; MPS, methyl phenyl sulfone; POPC, 1-
palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; HPLC, high-performance
liquid chromatography

Abstract

Clinical investigations have demonstrated a relationship between the extended use of rofecoxib and the increased risk for atherothrombotic events. This has led to the removal of rofecoxib from the market and concern over the cardiovascular safety of other cyclooxygenase (COX)-2 selective agents. Experimental findings from independent laboratories now indicate that the cardiotoxicity of rofecoxib may not be a class effect but because of its intrinsic chemical properties. Specifically, rofecoxib has been shown to increase the susceptibility of human low-density lipoprotein and cellular membrane lipids to oxidative modification, a contributing factor to plaque instability and thrombus formation. Independently of COX-2 inhibition, rofecoxib also promoted the nonenzymatic formation of isoprostanes and reactive aldehydes from biologic lipids. The basis for these observations is that rofecoxib alters lipid structure and readily forms a reactive maleic anhydride in the presence of oxygen. By contrast, other selective (celecoxib, valdecoxib) and nonselective (naproxen, diclofenac) inhibitors did not influence rates of low-density lipoprotein and membrane lipid oxidation. We have now further confirmed these findings by demonstrating that the prooxidant activity of rofecoxib can be blocked by the potent antioxidant astaxanthin in homochiral form (*all-trans* 3*S*, 3'*S*). These findings provide a mechanistic rationale for differences in cardiovascular risk among COX-selective inhibitors because of their intrinsic physicochemical properties.

Although cyclooxygenase (COX)-2 selective agents share a common pharmacologic target essential to prostaglandin biosynthesis, these compounds have otherwise distinct pharmacokinetic and pharmacodynamic properties that markedly affect their overall efficacy and safety. These

agents vary significantly in their rates of absorption, amount of protein binding, membrane locations, metabolism, and selectivity for their enzymatic target (Table 1). These pharmacokinetic differences influence efficacy, tolerance, tissue distribution, safety, and drug interactions. The structures of the 2 major classes of COX-2 inhibitors are also distinct (methylsulfone and sulfonamide) and account for important differences with respect to charge, lipophilicity, and susceptibility to oxidative modification. These chemical properties underlie other actions of these agents, as demonstrated in animal models of renal and vascular function, including inflammation.¹⁻³ These direct comparator studies showed a consistently worse outcome for rofecoxib with respect to cardiovascular activity, as compared with other COX-selective and nonselective agents.^{2,3}

	Celecoxib	Rofecoxib
Chemical structure		
Chemical family	Sulfonamide	Methylsulfone
COX-2 selectivity*	9	> 50
Charge at physiologic pH	Charged	Neutral
Membrane location	Hydrocarbon core	Headgroup region
Oral bioavailability (%)	22 to 40	92 to 93
Elimination half-life (h)	8 to 12	15 to 18
Volume of distribution (L)	≈ 400	≈ 90
Plasma protein binding (%)	> 97	86
Primary liver metabolism (cytochrome P450 enzymes)	Oxidation by CYP2C9, 3A4	Cytosolic reductase

*COX-2 selectivity based on the IC₈₀ (80% inhibitory concentration) of COX-2 relative to COX-1 using the William Harvey human modified whole blood assay.³⁵
 Table was modified from Chang IJ and Harris RC. *Hypertension*. 2005;45:178-180.

TABLE 1. Comparison of Celecoxib and Rofecoxib*COX-2 selectivity based on the IC₈₀ (80% inhibitory concentration) of COX-2 relative to COX-1 using the William Harvey human modified whole blood assay.³⁵Table was modified from Chang IJ and Harris RC. *Hypertension*. 2005;45:178-180.

As postulated by Nobel laureate John Vane in 2002, the basic differences in the physicochemical properties of the COX-2 inhibitors should be considered as an explanation for the increased cardiovascular risk associated with use of rofecoxib in an early clinical trial.^{2,3} This hypothesis has been subsequently validated by studies in independent laboratories, including one from E.J. Corey (Nobel laureate in chemistry, 1990). Reddy and Corey⁴ reported that rofecoxib readily formed a reactive metabolite, a maleic anhydride derivative (Fig. 1). This metabolite is capable of reactions with nucleophilic groups of various biologic molecules, especially amino groups, to cause disruption in essential cellular structure-function relationships. It is noteworthy that this reactive metabolite, formed in the presence of oxygen, could not be derived from other COX-2-selective agents, including celecoxib, valdecoxib, and lumoxicobin owing to differences in their chemical structure.

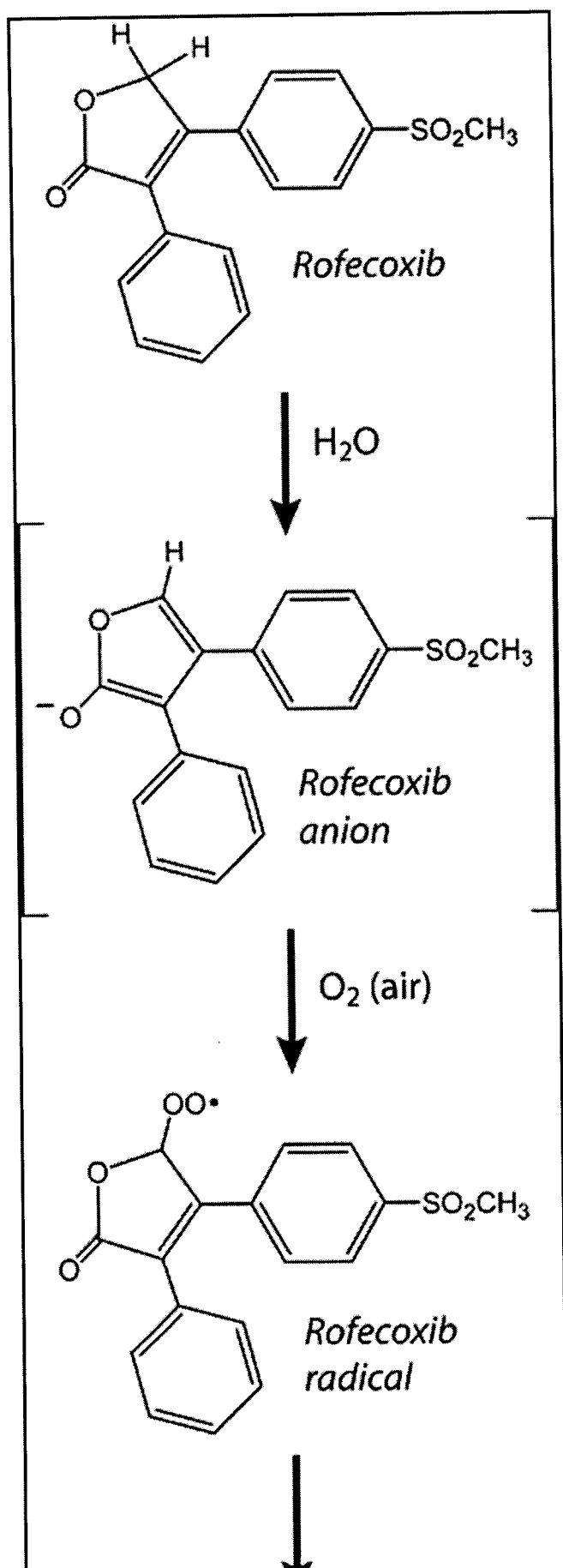


FIGURE 1. Chemical steps in the formation of a reactive metabolite for rofecoxib. Under physiologic conditions, rofecoxib readily ionizes to an anion that then forms a reactive maleic anhydride in the presence of oxygen.⁴ The metabolite is capable of reacting with polyunsaturated fatty acids associated with membrane phospholipid or LDL to form toxic lipid peroxy groups. The metabolite may also react with amino groups in proteins, thereby altering essential aspects of cellular structure and function.

The formation of a reactive metabolite from rofecoxib may specifically contribute to mechanisms of atherothrombotic disease. The maleic anhydride can form highly reactive peroxy radicals, thereby promoting lipid oxidation. As evidence for such a mechanism, we observed that rofecoxib, but not sulfonamide-type COX-2 inhibitors (celecoxib, valdecoxib), caused an increase in susceptibility of human low-density lipoprotein (LDL) and membrane lipid to oxidative modification.⁵ Consistent with the findings of Corey, the formation of isoprostanes and aldehydes with rofecoxib was nonenzymatic. In addition to a biochemical mechanism mediated by its metabolite, we observed that rofecoxib (and etoricoxib) altered the 3-dimensional structure of the lipid molecules, a process that could further accelerate the rate of lipid peroxidation.⁵ Again, these toxic effects of rofecoxib were not reproduced by other COX-selective or nonselective agents, including naproxen, ibuprofen, diclofenac, or meloxicam. These experimental findings could account for the cardiotoxicity associated with chronic rofecoxib use in various trials, including the Adenomatous Polyp Prevention on Vioxx (APPROVe) Trial.^{6,7} The APPROVe study led to the withdrawal of rofecoxib because it confirmed an unacceptable increase in cardiovascular events with this agent, independently of COX-1 inhibition with aspirin (so-called imbalance hypothesis) or blood pressure changes.⁷ As a logical extension of this work, we have now found that a potent, chain-breaking antioxidant, astaxanthin, can block these effects of rofecoxib in a highly reproducible manner.

MATERIALS AND METHODS

We tested the effects of COX selective and nonselective agents on rates of peroxidation in various lipid preparations with polyunsaturated fatty acids, including 1,2-diarachidonoyl-sn-glycero-3-phosphocholine (DAPC). These lipids along with cholesterol powder were obtained from Avanti Polar Lipids (Alabaster, AL) and characterized by high-performance liquid chromatography. Astaxanthin (all-*trans* 3S, 3'S; chiral purity >97%) was synthesized by Synchem, Inc (Des Plaines, IL; patents pending) and supplied by Hawaii Biotech, Inc. Other chemical reagents and all drugs were purchased from independent commercial sources, including Sigma (St Louis, MO), Acros Organics (Morris Plains, NJ), and Calbiochem (San Diego, CA). Depending on their solubility properties, drugs and sulfone compounds (methyl phenyl sulfone [MPS], dimethyl sulfone) in powder form were solubilized in organic solvents before adding to buffer at low volumes. Lipids were stored at -80°C in high-performance liquid chromatography-grade chloroform.

LDL Isolation and Oxidation Analysis

The effects of the compounds on lipid peroxidation were tested in isolated human LDL. Human venous blood was collected into vacutainer tubes containing K₃-ethylenediaminetetraacetic acid after a 12-hour fast. Plasma was immediately separated by centrifugation at 3000g for 25 minutes. LDL was obtained from plasma by ultracentrifugation using a procedure similar to that

reported by Chung et al⁸ using discontinuous KBr gradient. The purity of the freshly isolated LDL was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using the procedure of Laemmli.⁹

The extent of oxidation was measured for the LDL in the absence and presence of the drugs over a range of concentrations. Malondialdehyde (MDA), a product of lipid oxidation, was measured by the reaction of this aldehyde with thiobarbituric acid in an acid medium to form a stable chromogen, referred to as thiobarbituric acid-reactive substances.¹⁰ Purified LDL (100 µg protein/mL) was incubated for 30 minutes with either vehicle or nonsteroidal anti-inflammatory drug (NSAID) at various concentrations followed by the addition of 50 µmol/L CuSO₄ at 37°C. Conjugated diene formation was measured by continuously monitoring the change in absorbance at 234 nm on a Beckman DU 640 spectrophotometer, as described by Esterbauer et al.¹¹ Stock solutions were tested for iron contamination, which can contribute independently to lipid peroxidation and assay artifacts.¹²

Oxidation Analysis With Astaxanthin

The ability of astaxanthin to block the prooxidant effects of rofecoxib was tested in lipid vesicles reconstituted from DAPC (10 mg/mL) in physiologic buffer (0.5 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 154 mmol/L NaCl, pH=7.32). The lipid vesicles also contained cholesterol at a level that reproduced physiologic conditions (0.2 cholesterol-to-phospholipid mole ratio). The drug effects were tested in the vesicles after addition of astaxanthin, rofecoxib, or the combination of these 2 agents at an identical concentration (250 nmol/L). The lipid peroxidation reaction occurs gradually under normal atmospheric oxygen conditions in a shaking water bath (37°C). To measure lipid hydroperoxide (LOOH) formation, we used an assay that measures the conversion of I₂ to I₃⁻ (tri-iodide). This reaction takes place in the presence of LOOH in a manner that can be measured photometrically at 365 nm.¹³ This assay is sensitive to peroxide concentrations as low as 10 µmol/L and has the further advantage in that it does not require the use of exogenous peroxide radical initiators.

Determination of F₂-isoprostanes by Gas Chromatography (GC) With Negative Chemical Ionization Mass Spectroscopy

F₂-isoprostanes are derived principally from the formation of positioned peroxy radical isomers of arachidonic acid, endocyclization to prostaglandin G₂-like structures, and reduction to prostaglandin F₂-like compounds. Total levels of F₂-isoprostanes, in DAPC lipid vesicles prepared in the presence of vehicle or drug, were measured by GC-mass spectroscopy (MS) with negative chemical ionization as described by Walter et al.¹⁴ Peroxidation of lipids occurred over time in the absence of any exogenous initiators at 37°C. F₂-isoprostane formation was also measured independently using MS in a blinded study at the Antioxidant Research Laboratory, Tufts University, Boston, MA.

Oxygen Radical Absorption Capacity (ORAC) of Human Plasma

The comparative effect of COX-2 selective agents on the antioxidant capacity of human

plasma was assessed using the ORAC assay. This assay was carried out according to the method of Huang et al¹⁵ with fresh plasma using a microplate fluorescence reader in 96-well format with the excitation and emission filters set at 485 and 530 nm, respectively.

Preparation of Lipid Vesicles

The effects of selective and nonselective COX inhibitors on membrane molecular structure and rates of lipid peroxidation were assessed in multilamellar lipid vesicles. Multilamellar lipid vesicles were prepared in buffer (0.5 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and 154.0 mmol/L NaCl, pH=7.2) and by the method of Bangham.¹⁶ For x-ray diffraction analysis, the final phospholipid (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) concentration was 2.5 mg/mL and the mole ratio of cholesterol to phospholipid was 0.2:1. The diffraction studies used a mole ratio of drug to phospholipid of 1:10, resulting in a final concentration of <5%, by mass. Membrane samples were oriented for x-ray diffraction and analyzed, as previously described in detail.^{5,17}

Small-angle X-ray Diffraction Analysis of Drug/Lipid Structure

Small-angle x-ray diffraction approaches were used to examine the effects of COX-2 inhibitors on the time-averaged molecular structure of lipids in vascular cell-like membranes. X-ray diffraction experiments were conducted by aligning the samples at grazing incidence with respect to a collimated x-ray source. Corrected diffraction orders obtained from samples in this study were analyzed using Fourier summation to yield 1-dimensional electron density profiles (\AA vs. electrons/ \AA^3) of the membrane lipid bilayer. A detailed explanation of membrane diffraction analysis was described previously.¹⁸

Statistical Analysis

Data are presented as mean \pm SD. The significance of differences between results from independent experimental conditions (conducted in triplicate) was tested using the 2-tailed Student *t* test. A value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Rofecoxib Increases the Susceptibility of Human LDL to Oxidative Modification: Comparison to Other COX-2 Inhibitors and NSAIDs

Minimally modified or oxidized LDL has an essential role in atherosclerotic plaque instability by contributing to mechanisms of endothelial dysfunction and inflammation.^{19,20} Oxidative LDL contributes directly to foam cell formation, endothelial dysfunction, and destructive inflammatory processes associated with plaque instability and thrombus formation.^{21,22} In vivo studies show that levels of oxidized LDL strongly correlate with the severity of acute coronary syndromes and plaque instability.^{23,24} In a prospective study of more than 600 patients, we observed that levels of lipid oxidation markers were highly predictive of coronary events over a 3-year period, independently of traditional risk factors and markers of inflammation.²⁵ Thus, a prooxidant effect with rofecoxib may be an important clue to our understanding the basis for its toxicity and lead to pharmacologic approaches to block this activity.

We evaluated the effects of rofecoxib on rates of lipid peroxidation in isolated human LDL and lipid vesicles enriched with polyunsaturated fatty acids (eg, arachidonic acid). Lipid peroxidation in these various biologic preparations was monitored and compared with another selective (celecoxib) and nonselective (naproxen, diclofenac) COX inhibitors under identical conditions. The activity of rofecoxib was also compared with sulfone analogs, including MPS and dimethyl sulfone (data not shown).

After incubation with human LDL, rofecoxib significantly ($P < 0.001$) *decreased* the lag time for human LDL conjugated diene formation by $42.8\% \pm 1.5\%$ at 100 nmol/L (Fig. 2). This pronounced effect on the rate of conjugated diene formation indicates that rofecoxib has potent prooxidant activity, as evidenced by depleted LDL antioxidant capacity. In addition to measuring conjugated diene formation, we also measured the formation of reactive aldehydes, especially MDA. Consistent with its effect on conjugated diene formation, rofecoxib and etoricoxib also caused marked increases in MDA levels. The comparative effects of these agents on MDA formation from human LDL (measured as thiobarbituric acid-reactive substances) are reported in Figure 2. Compared with celecoxib, naproxen, and diclofenac, only rofecoxib caused a significant increase in MDA levels, even at a concentration (50 nmol/L) that was 10-fold lower than that used for comparison drugs (50 nmol/L). Prooxidant changes in conjugated diene formation or MDA levels were not observed after treatment with other COX selective or nonselective inhibitors under identical conditions. Additionally, other sulfone-containing compounds (MPS, dimethyl sulfone) had no effect on LDL oxidation (data not shown). As compared with vehicle-treated LDL samples (MDA level of 3.23 ± 0.28 $\mu\text{mol/L}$), there were no significant changes in LDL oxidation for samples treated with either dimethyl sulfone (3.32 ± 0.19 $\mu\text{mol/L}$, $P = 0.9$) or MPS (3.25 ± 0.13 $\mu\text{mol/L}$, $P = 0.7$) at a concentration of 1 $\mu\text{mol/L}$.

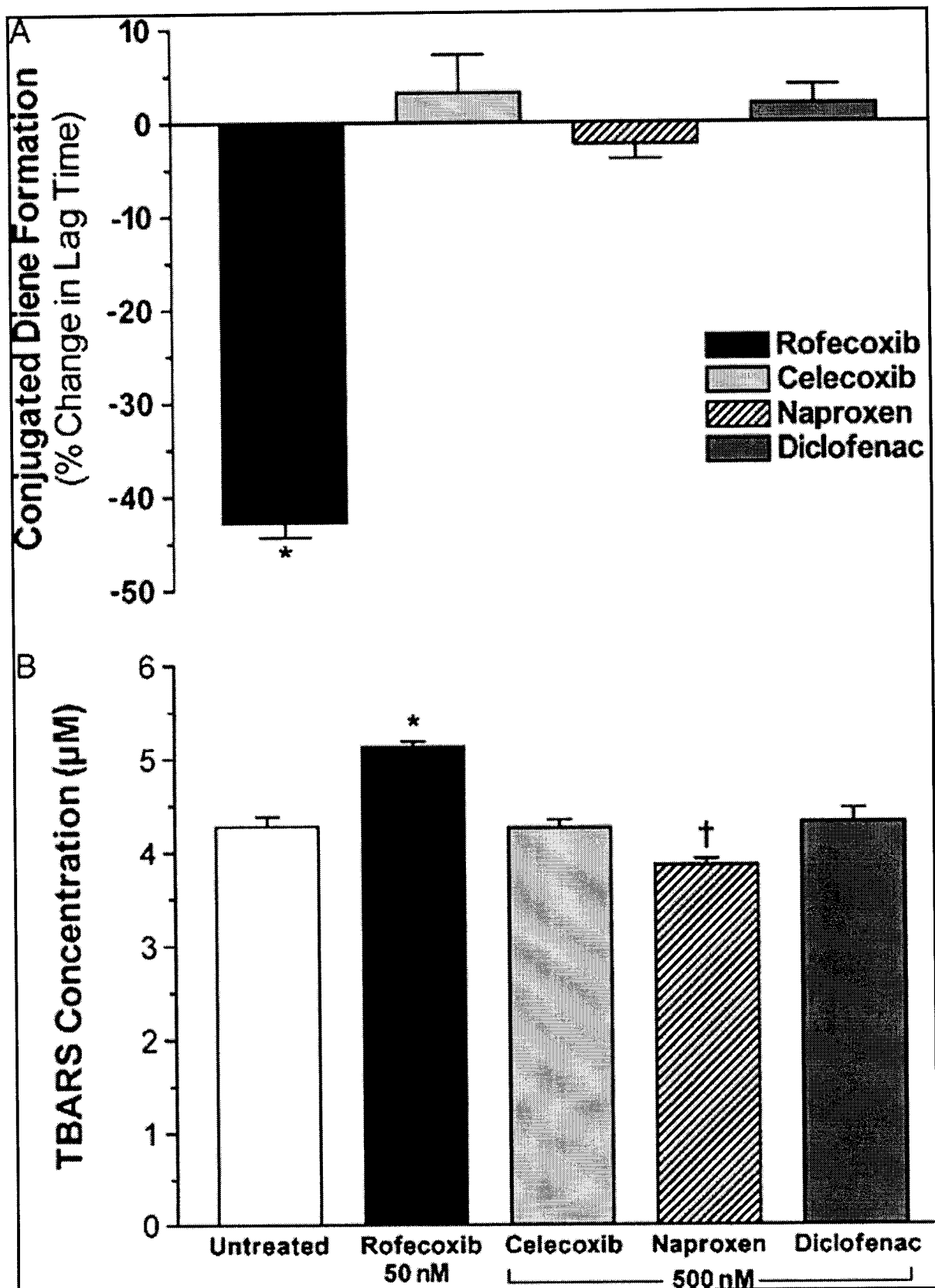


FIGURE 2. A, Comparative effects of NSAIDs on rates of conjugated diene formation in human LDL (100 µg protein/mL) after incubation with COX-2 selective (celecoxib, rofecoxib) and nonselective inhibitors (diclofenac, naproxen). Diene formation in the presence of each agent at a concentration of 100 nmol/L was monitored at 234 nm and compared with vehicle (control). The

lag time was calculated from the intercept of the lines drawn through the linear portions of the lag phase and propagation phase. LDL oxidation was initiated with CuSO_4 at 37°C . Values are mean \pm SD of experiments done in triplicate. * $P<0.001$, different from vehicle-treated LDL samples. B, Comparative effects of NSAIDs on formation of thiobarbituric acid-reactive substances in human LDL (100 μg protein/mL) after incubation with COX-2 selective (celecoxib, rofecoxib) and nonselective inhibitors (diclofenac, naproxen, ibuprofen) at 500 nmol/L. Oxidation was initiated with CuSO_4 at 37°C . The effects of the drugs on LDL oxidation were compared with vehicle (control) based on thiobarbituric acid-reactive substances formation measured at an absorbance of 532 nm. * $P<0.001$ and † $P<0.01$ versus vehicle treated samples. (Figure modified from reference 5, with permission from Elsevier.)

Effect of COX-2 Inhibitors and NSAIDs on Isoprostane Formation from Membrane Phospholipids

Isoprostanes are prostaglandin isomers that can be generated nonenzymatically by free-radical modification of arachidonic acid associated with phospholipid in LDL and cellular membranes. F_2 -isoprostanes have been specifically identified in atherosclerotic plaques where they are mediators of inflammation.²⁶ We tested the effects of these agents on peroxidation of lipid vesicles containing arachidonic acid, the substrate for nonenzymatic formation of isoprostanes. Using mass spectroscopy, we observed pronounced differences in isoprostane generation among the COX-2 inhibitors. Levels of isoprostanes were shown to increase from 140 ± 25 ng/100 $\mu\text{mol/L}$ (mean \pm SD) in vehicle-treated samples to 190 ± 18 ng/100 μL ($P<0.0025$) and 224 ± 22 ng/100 μL ($P<0.0001$) in the presence of 100 nmol/L rofecoxib and etoricoxib, respectively (Fig. 3A). By contrast, celecoxib had no significant effect on peroxidation of arachidonic acid-enriched vesicles, even at higher concentrations (data not shown).

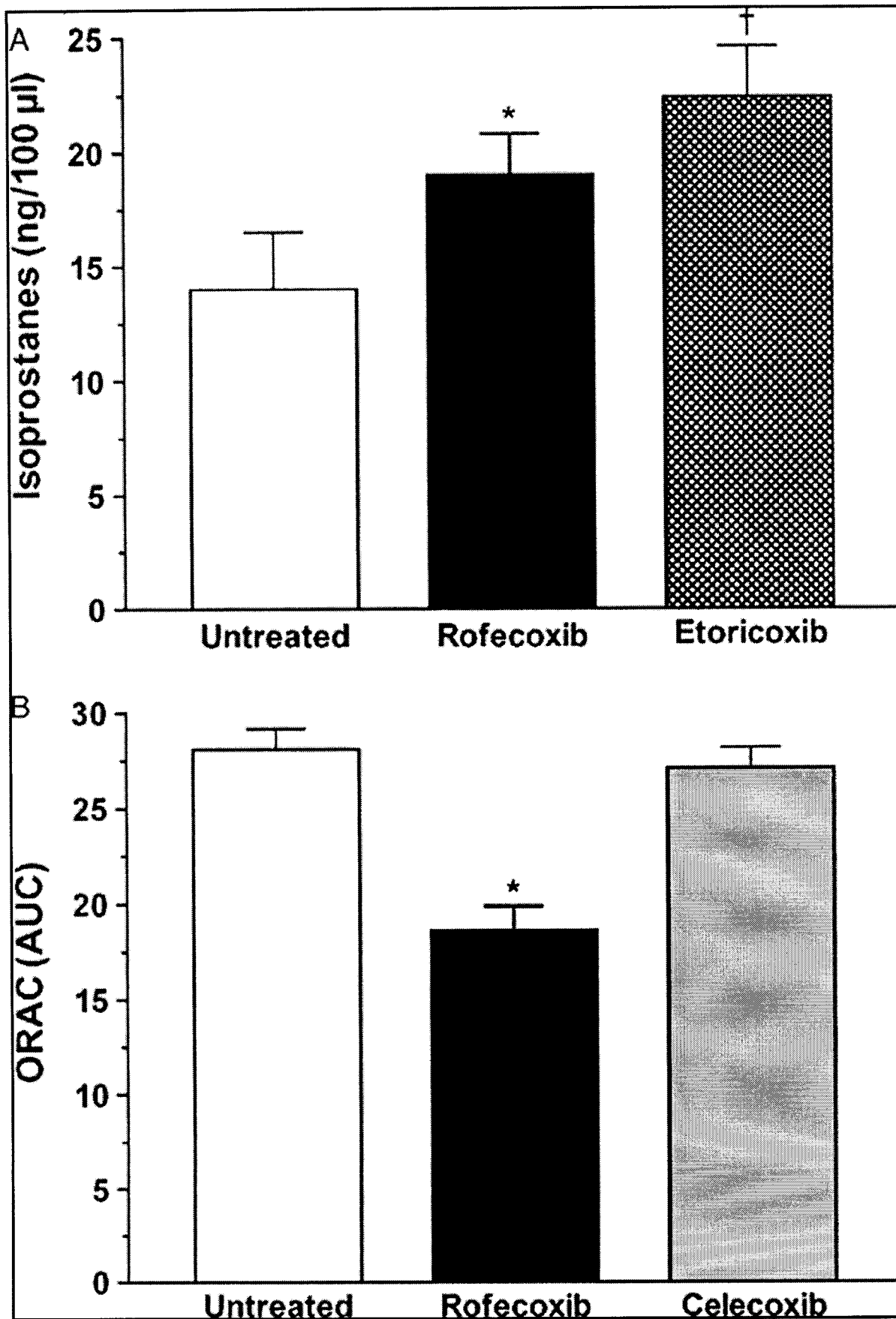


FIGURE 3. A, Effects of rofecoxib and etoricoxib (100 nmol/L) versus vehicle treatment on

isoprostane formation from lipid vesicles enriched with arachidonic acid (0.25 mg/mL). Peroxidation of lipids occurred over time in the absence of any exogenous initiators at 37°C. Total levels of F₂-isoprostanes were measured by GC-MS with negative chemical ionization as described by Walter et al 14 in DAPC lipid vesicles prepared in the presence of vehicle or drug. Values are mean±SD (n=4), *P<0.01 and †P<0.0001 versus vehicle-treated samples. B, Comparative effects of COX-2 inhibitors rofecoxib and celecoxib on the antioxidant capacity of human plasma were assessed using the ORAC assay. The area under the curve (AUC) from the analysis was reported as a function of treatment at 1.0 µmol/L. Plasma oxidation was initiated with AAPH at 37°C. The effects of the drug were compared with vehicle (control). *P<0.0001, rofecoxib versus either vehicle- or celecoxib- treated samples. (Figure modified from reference 5, with permission from Elsevier.)

Effect of Rofecoxib and Celecoxib on ORAC of Human Plasma

The comparative effects of rofecoxib and celecoxib on the antioxidant capacity of human plasma were assessed using the ORAC assay.¹⁵ The area under the curve from the ORAC analysis is reported in Figure 3B. Consistent with a prooxidant effect, rofecoxib significantly (*P*<0.001) reduced the ORAC value by 34% (28.1±1.2 in vehicle-treated samples to 18.6±1.3) at 1.0 µmol/L. In parallel experiments, celecoxib did not significantly change this value, even at the highest concentration tested (10.0 µmol/L), in which the ORAC value was 28.5±0.1.

As reported in the *Physicians Desk REFERENCE* (2003), the maximum plasma concentration (*C*_{max}) for celecoxib at an approved 200-mg dose is 1.85 µmol/L (705 ng/mL). In the case of rofecoxib, an approved dose of 25 mg results in a plasma concentration of 658 nmol/L (207 ng/mL). In the case of astaxanthin, a 100-mg oral "racemic" dose results in a maximum plasma concentration of 2.18 µmol/L (1.3 mg/L).²⁷ Thus, the effects we are reporting with these drugs and astaxanthin at nanomolar to low micromolar concentrations are pharmacologically relevant.

Inhibition of Lipid Peroxidation with Rofecoxib by Astaxanthin

Astaxanthin (3,3'-dihydroxy-[beta],[beta]'-carotene-4,4'-dione) is a carotenoid with significant antioxidant activity, even as compared with other compounds in this class.²⁸⁻³⁰ This highly lipophilic molecule reduces oxidative damage to biologic lipids by quenching singlet oxygen and scavenging free radicals. The chemical basis for its activity is a 40-carbon polyene chromophore terminated by cyclic end groups with oxygen-containing polar substituents. In addition to their role in electron stabilization during the scavenging process, the polar terminal groups allow for a preferred membrane orientation that facilitates its scavenging properties.³⁰ It has no pro-vitamin A activity in mammals.³¹ By contrast, [beta]-carotene and other analogs (lycopene) that do not possess these oxygenated rings are not as well organized in the membrane-lipid bilayer and show inferior antioxidant activity under identical conditions (data not shown).

As shown in Figure 4, the addition of rofecoxib alone at 250 nmol/L separately caused a significant 7.4% increase (*P*<0.05 vs control) in lipid peroxide levels in vesicles enriched with polyunsaturated fatty acids (arachidonic acid), consistent with our measurements of isoprostane formation. The effect of rofecoxib on lipid peroxidation was assessed after a 10-hour incubation period at 37°C and the control level of LOOH formation was 750 µmol/L. By contrast, addition of astaxanthin alone at this same concentration produced an opposite, antioxidant effect as

evidenced by a 4.4% decrease ($P < 0.05$ vs control) in lipid peroxidation. Remarkably, astaxanthin was able to completely inhibit the adverse effects of rofecoxib on lipid peroxidation when added together with rofecoxib at an equimolar concentration (decrease in lipid peroxidation of 6.5%; $P < 0.05$ vs control).

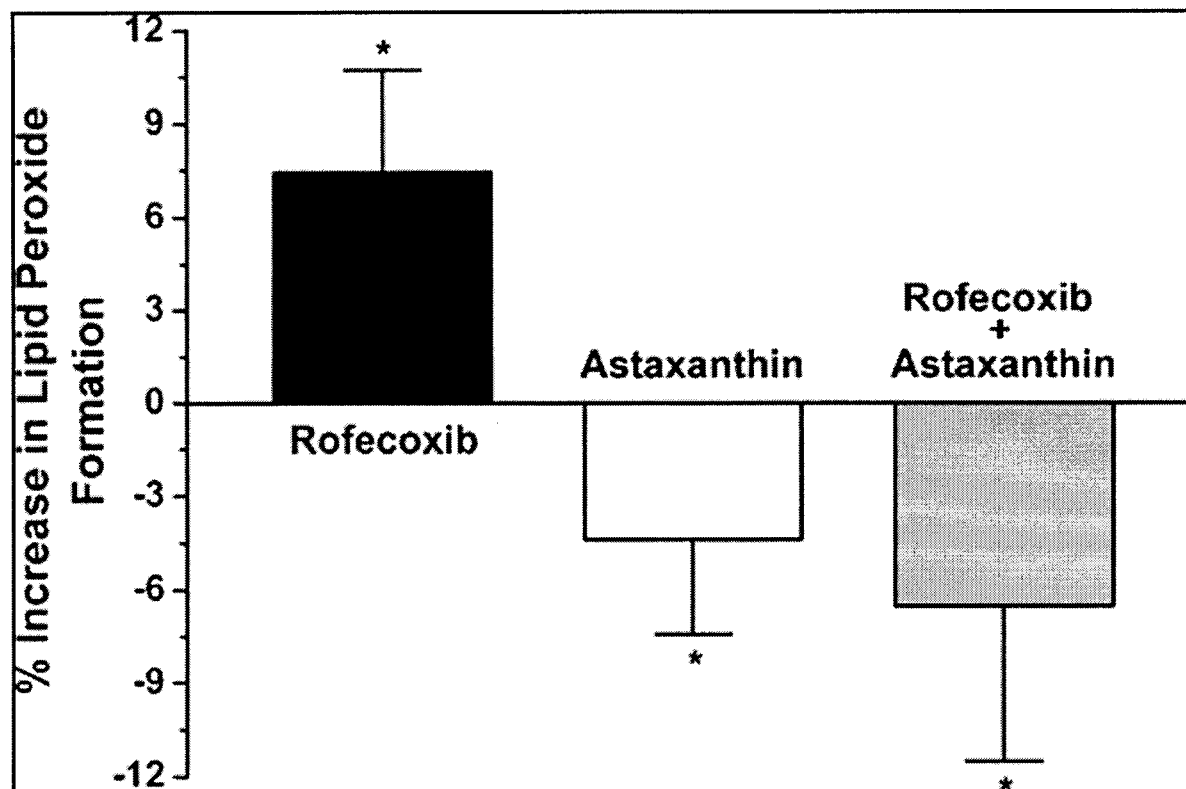


FIGURE 4. Separate versus combined effects of rofecoxib and astaxanthin on LOOH formation in lipid vesicles enriched with arachidonic acid at a concentration of 250 nmol/L. Formation of LOOH was measured at 365 nm as the conversion of I_2 to I_3^- (tri-iodide) after incubation for 10 hours at 37°C. Values are mean \pm SD of 6 separate experiments. * $P < 0.05$, for all treatments as compared with control.

Effect of COX-2 Inhibitors on Lipid Structure

To understand how the distinct properties of the COX-2 inhibitors influence the susceptibility of LDL and membrane lipids to oxidative modification, we used small-angle x-ray diffraction approaches.³² The addition of rofecoxib produced a change in electron density consistent with a location in the phospholipid headgroup, as we previously demonstrated.⁵ At the same time, rofecoxib caused disordering in the hydrocarbon core, similar to thermal heating or oxidative damage to the membrane.^{33,34} Thus, the prooxidant activity of rofecoxib may be related, in part, to physicochemical changes in lipid structure. By contrast, the addition of celecoxib to the phospholipid bilayer was associated with the hydrocarbon core, a location attributed to its greater lipophilicity. These differences in the molecular membrane interactions of the COX-2 inhibitors may contribute to their distinct physicochemical effects on lipid peroxidation (Fig. 5).

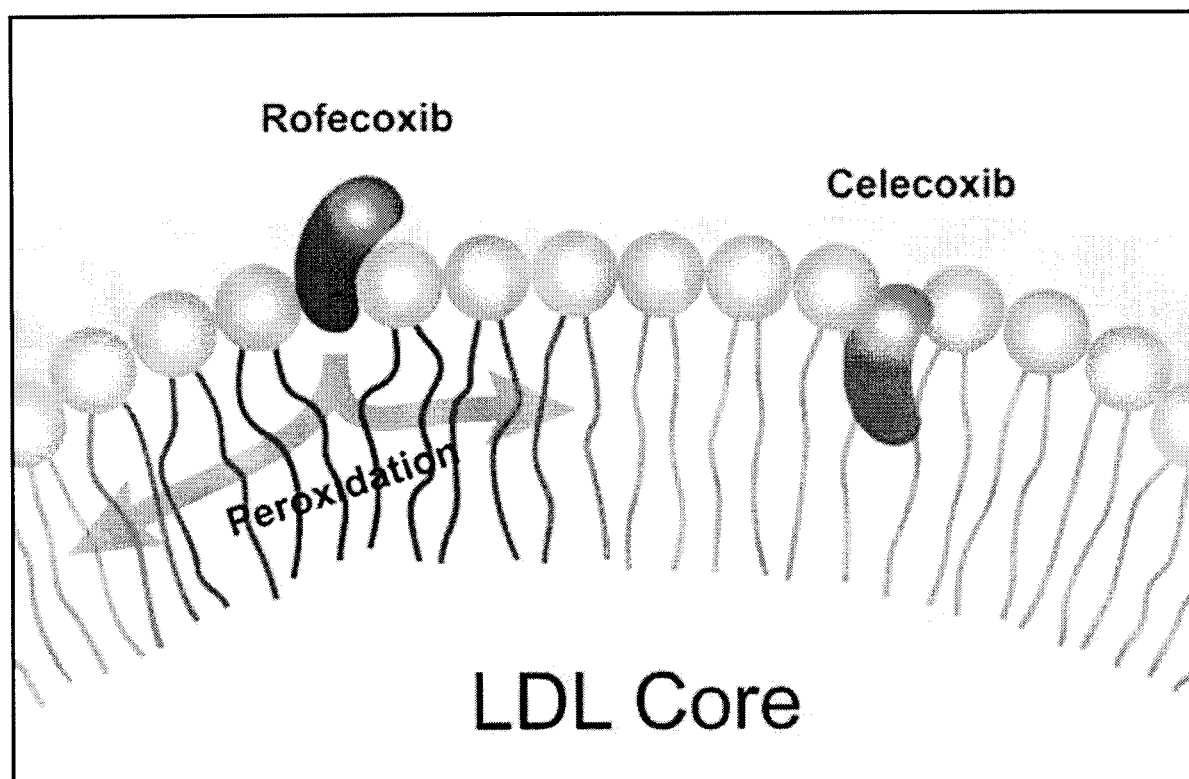


FIGURE 5. Schematic illustration of structural changes in membrane lipids with rofecoxib, on the basis of small angle x-ray diffraction analysis. The location of rofecoxib in the phospholipid headgroup region caused a disordering of the phospholipid acyl chains. The alteration in the intermolecular packing of the lipid molecules may facilitate the diffusion of free radicals. By contrast, celecoxib had a well-defined location in the membrane hydrocarbon core; a position consistent with its highly lipophilic properties. The equilibrium position of the celecoxib molecule did not cause a disordering in the lipid molecules.

CONCLUSION

We have reviewed a mechanistic basis for cardiotoxicity with rofecoxib that can be attributed to its distinct chemical properties and prooxidant activity (Fig. 6). Rofecoxib readily forms a highly reactive maleic anhydride derivative capable of reacting with various biologic targets, including arachidonic acid in phospholipids, to form atherogenic aldehydes and isoprostanes. We have also now demonstrated a pharmacologic approach to block the prooxidant effects of rofecoxib using a highly lipophilic chain-breaking antioxidant, astaxanthin. Collectively, these findings indicate that rofecoxib is cardiotoxic because of inherent chemical properties that are unrelated to COX-2 inhibition.

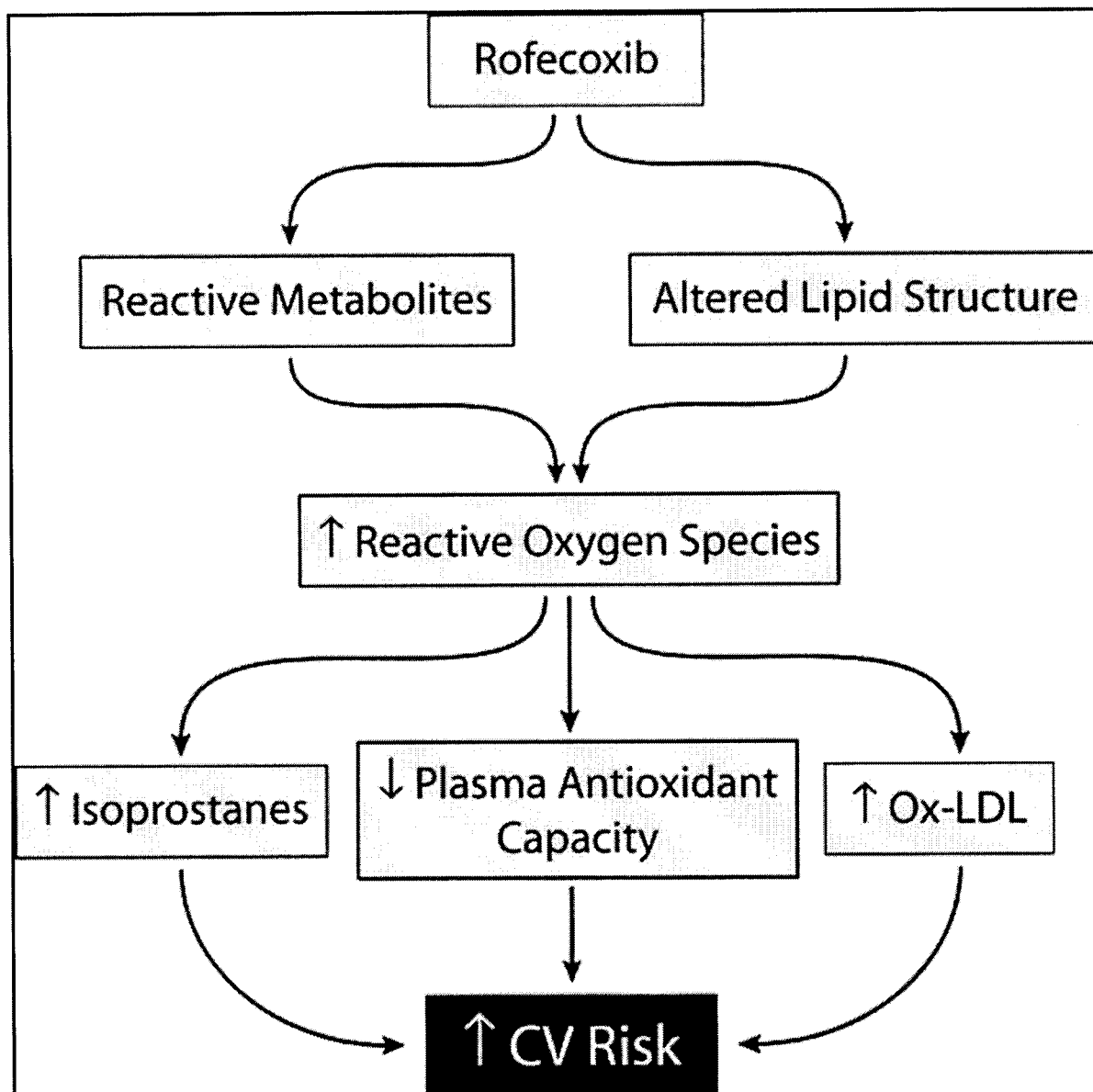


FIGURE 6. A summary of the cardiotoxic mechanism for rofecoxib based on experimental findings.^{4,5} The rofecoxib molecule alters lipid structure and undergoes conversion to a reactive metabolite that may stimulate formation of reactive oxygen species, such as lipid peroxy groups. As evidence of this prooxidant activity, rofecoxib increased levels of isoprostanes, oxidized LDL, and reduced plasma antioxidant capacity. These oxidized lipids contribute to endothelial dysfunction and inflammation, thereby contributing to mechanisms of atherothrombosis.

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