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INFLUENCE OF CARDIOPROTECTIVE CYCLOOXYGENASE AND LIPOXYGENASE INHIBITORS ON PEROXIDATIVE INJURY TO MYOCARDIAL-MEMBRANE PHOSPHOLIPID

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Abstract—Oxygenase-catalyzed and non-enzymatic polyunsaturated fatty acid peroxidations have potential pathogenic roles in ischemic-reperfusion damage to the myocardium. Certain oxygenase inhibitors protect heart muscle from irreversible ischemic injury, and some antiperoxidants can inhibit oxygenase enzymes. We investigated the antiperoxidative abilities of eight anti-ischemic, cardioprotective oxygenase inhibitors to prevent myocardial-membrane phospholipid peroxidation through superoxide-driven, iron-promoted reactions with xanthine oxidase as the source of superoxide. Flurbiprofen, ibuprofen, and REV-5901-5 did not affect peroxidation at concentrations up to 1000 μM. BW755C, AA-861, nafazatrom, dipyridamole, and propyl gallate did protect and cardiac lipids against oxidative injury in a concentration-dependent manner with respective and antiperoxidant IC_{50} values (concentrations at which peroxidation was inhibited by 50%) of 0.22, 1.25, 3.0, 3.6 and 50 μM. Catechin and phenidone, known oxygenase inhibitors not yet evaluated as anti-ischemic agents, were also found to be antiperoxidants at low micromolar concentrations. Four cyclooxygenase inhibitors ineffective against myocardial infarction (aspirin, indomethacin, naproxen, and sulfinpyrazone) evidenced no antiperoxidant properties at concentrations up to 500 μM. The oxygenase inhibitor-antiperoxidants identified could neither quench superoxide radical nor inhibit xanthine oxidase.However, they were able to interrupt the propagation of an on-going peroxidation reaction. Their antiperoxidant profiles resembled those of known antioxidants, such as α-tocopherol, which inhibit peroxidation by intercepting lipid free-radical intermediates. These data raise the possibility that at least some oxygenase inhibitors could exert cardioprotective effects by directly influencing the sensitivity of myocardial-membrane phospholipid to peroxidative injury. Consequently, recognition of the antiperoxidant properties of these agents may aid dissection of their physiological and pharmacological actions.

The synthesis of a variety of potent lipid mediators, collectively termed eicosanoids, is dependent upon two types of heme-containing oxygenases which catalyze the introduction of molecular oxygen into polyunsaturated fatty acid (PUFA†) substrate, notably arachidonic acid [1]. Cyclooxygenase, a component of prostaglandin H synthetase, catalyzes PUFA bis-dioxygenation to yield endoperoxide precursors of prostaglandins, thromboxanes, and prostacyclin [2]. Lipoxygenase catalyzes PUFA monodioxygenation to allylic hydroperoxides, some of which are precursors to leukotriene fatty-epoxides, glutathione conjugates, and lipoxins [3]. Since the eicosanoid products of cyclooxygenase and lipoxygenase catalysis exert profound pathophysiological effects in a variety of disease states [4], compounds which inhibit these oxygenases, and consequently attenuate production of fatty-peroxide eicosanoid precursors, have wide pharmacological interest [5, 6] as, for example, potential anti-inflammatory drugs [7].

Leukocyte- and platelet-derived eicosanoids are believed to exacerbate irreversible ischemic heart-muscle damage and promote the development of arrhythmias during reperfusion [8–10]. Because the extent of myocardial necrosis (i.e. infarction) determines subsequent mortality [11], various cyclooxygenase and lipoxygenase inhibitors have been studied for their abilities to reduce infarction in animal models of occlusion-reperfusion cardiac injury. Those which have consistently salvaged jeopardized myocardium are the cyclooxygenase inhibitor flurbiprofen [12]; the lipoxygenase inhibitors AA-861 [13], dipyridamole [14], ibuprofen [15], and REV-5901-5 [16]; and the "dual inhibitors" BW755C [17, 18], nafazatrom [19–21], and propyl gallate [22]. Some cyclooxygenase inhibitors, aspirin [23], indomethacin [24], naproxen [25], and sulfinpyrazone [25], do not reduce the extent of experimentally-induced myocardial infarction. Other compounds, such as the dual inhibitors catechin [26] and phenidone [27], have yet to be studied in occlusion-reperfusion cardiac-injury models.

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† Abbreviations: PUFA, polyunsaturated fatty acid; O_2^-, superoxide anion radical; XOD, xanthine:oxygen oxidoreductase, xanthine oxidase (EC 1.2.3.2); HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; TBA, thiobarbituric acid; ADP, adenosine diphosphate; HX, hypoxanthine; BHT, butylated hydroxytoluene; SOD, superoxide dismutase; and MDA, malondialdehyde.

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Aside from the eicosanoid end-products of enzymatic arachidonic acid oxygenation, free radicals containing oxygen are believed to be another causal contributor to myocardial ischemic-reperfusion injury [28, 29]. Superoxide (O_2^-)-dependent, iron-mediated peroxidation of PUFAs esterified to myocardial-membrane phospholipid, with xanthine oxidase (XOD) as a potential O_2^- source [30], may induce irreversible cardiac ischemic [31] and reperfusion [32] damage. Antioxidants have been shown to reduce infarction in some animal models of myocardial ischemia [29, 33].

Cyclooxygenase and lipoxygenase enzymes can be inhibited by antioxidants [5, 6], and their catalytic mechanism has many of the characteristics of non-enzymatic, free radical-dependent, iron-mediated lipid peroxidation [2, 3]. Consequently, although complete description of cyclooxygenase and lipoxygenase catalytic intermediates awaits further study, reaction mechanisms for these metalloenzymes invoke participation of their iron-redox centers in the initiation of PUFA peroxidation as a controlled, radical-dependent chain reaction [2–6]. In contrast to non-enzymatic PUFA auto-oxidation, however, the oxygenase-mediated formation of fatty endo- and hydro-peroxides is stereospecific and displays positional oxygenation [34].

The sensitivity of oxygenase-catalyzed and non-enzymatic PUFA peroxidation to antioxidants and the pathogenic roles which both types of lipid peroxidation may have in myocardial ischemic-reperfusion damage prompted us to evaluate, quantitatively, the abilities of eight cardioprotective cyclooxygenase and lipoxygenase inhibitors to protect myocardial membrane phospholipid PUFAs from O_2^--dependent, iron-mediated free radical injury in a physiologically-relevant [32] peroxidation system with XOD as a O_2^- source. The resultant data are placed in the comparative context of the anti-peroxidative properties of four other oxygenase inhibitors shown not to reduce infarction. The investigations reported offer the first direct analysis of these agents as inhibitors of radical-induced injury to myocardial-membrane phospholipid and indicate that the oxygenase inhibitor-antiperoxidants indentified act as "chain-breaking" molecules.

MATERIALS AND METHODS

Materials. *N*-2-Hydroxyethylpiperazine-*N*′-ethanesulfonic acid (HEPES), tris(hydroxymethyl)aminomethane (Tris), thiobarbituric acid (TBA), adenosine diphosphate (ADP), xanthine, hypoxanthine (HX), butylated hydroxytoluene (BHT), EDTA, propyl gallate, catechin, phenidone, indomethacin, naproxen, and aspirin (acetylsalicylic acid) were from the Sigma Chemical Co. (St Louis, MO). XOD (analytical preparation from bovine milk; 1 unit/mg), superoxide dismutase (SOD; superoxide:superoxide oxidoreductase, EC 1.15.1.1; analytical preparation from bovine erythrocytes; 5000 units/mg), and ferricytochrome *c* (horse-heart) were from Boehringer-Mannheim (Indianapolis, IN). BW755C was obtained from Wellcome Research Laboratories (Beckenham, U.K.). AA-861 was from Takeda Chemical Industries Ltd. (Osaka, Japan). Nafazatrom (BAY g 6575) was from Miles Laboratories (West Haven, CT). Dipyridamole and ibuprofen were from Lederle Laboratories (Wayne, NJ). Flurbiprofen was from Upjohn (Kalamazoo, MI). REV-5901-5 was from Revlon (New York, NY), and sulfinpyrazone from Ciba-Geigy (Summit, NJ). Solvents were of analytical grade as purchased (Burdick & Jackson, Muskegon, MI). Water was purified with a Milli-Q system (Millipore, Bedford, MA).

Peroxidation reaction system. Liposomes were prepared from purified rat myocardial-membrane phospholipid as detailed [35]. Lipid was quantified as lipid phosphate [36]. The phospholipids were subjected to O_2^--dependent, iron-mediated peroxidation at 37° in a reaction system containing 10 mM HEPES–0.145 M KCl, pH 7.4; 0.1 mM Fe^{3+}–1.0 mM ADP complex; 1.0 mM HX; 10 mUnits XOD/ml; and 125 μg cardiac phospholipid/ml. An oxygenase inhibitor was introduced into some peroxidation reactions either just prior to the start of the reaction or during an on-going reaction and at the concentrations specified in the text and in the legends to Figs. 2 and 3.

Production of O_2^- by the XOD and HX components of the peroxidation reaction system. The kinetics of O_2^- formation from XOD + HK were monitored spectrophotometrically at 550 nm as the SOD-inhibitable reduction of ferricytochrome *c*. The assay mixture, at 37°, contained: 10 mM HEPES–0.145 M KCl, pH 7.4; 1.0 mM HX; 10 mUnits XOD/ml; and 76 μM ferricytochrome *c*. The extinction coefficient 21/mM/cm was used to calculate the amount of O_2^- produced [37].

Conjugated diene analysis. For conjugated diene analysis, lipids were extracted out of the peroxidation reaction mixture and a parallel, nonperoxidized lipid sample [35]. The purified lipids were dissolved in 1.0 ml spectro-grade cyclohexane. Spectra of the lipids were taken against cyclohexane from 190 nm to 400 nm in a DU-7 kinetic spectrophotometer (Beckman, Palo Alto, CA). The nonperoxidized lipid spectrum was considered the blank spectrum. A difference spectrum was obtained between the peroxidized sample and the blank sample, and net conjugated diene formation was calculated from the difference spectrum with the molar absorptivities detailed [35].

Quantification of lipid peroxidation. Peroxidation was terminated by adding 0.15 ml of ice-cold 76% (w/v) trichloroacetic acid in 2.3 N HCl to each milliliter of peroxidation reaction. Lipid peroxide formation and decomposition were measured as thiobarbituric acid (TBA)-reactive material [35]. Under these conditions, TBA-reactive material largely reflects the malondialdehyde (MDA) produced from O_2^--dependent, iron-promoted cardiac phospholipid peroxidation [35]. The test substances studied did not influence the TBA-reactivity of purified MDA and were not themselves TBA-reactive. Concentration–response curves were generated with the assistance of RS/1 software (BBN Corp., Cambridge, MA) on an IBM PC-AT (IBM Corp., Boca Raton, FL).

Assessment of O_2^- trapping. Prevention of the SOD-inhibitable reduction of ferricytochrome *c* by

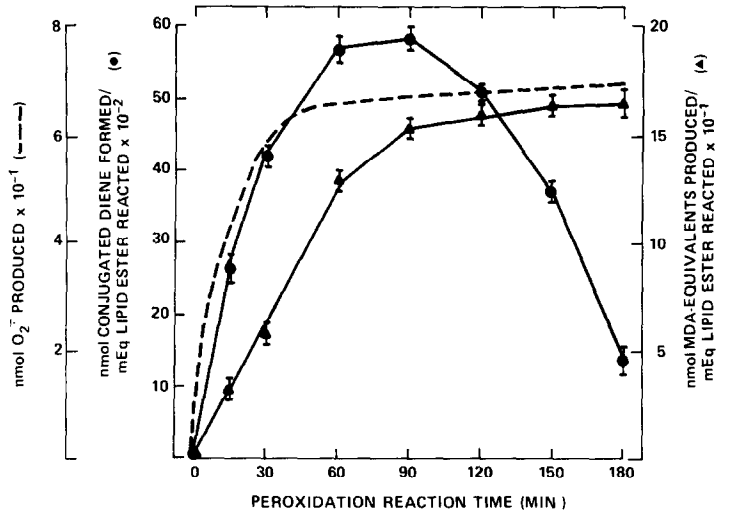

Fig. 1. Time-courses of O_2^- production and O_2^--, iron-dependent myocardial-membrane phospholipid peroxidation. Formation of O_2^- from 1.0 mM HX and 10 mUnits XOD/ml was assessed spectrophotometrically as the reduction of ferricytochrome *c* and is represented by the kinetic spectrophotometric trace given (– –). Peroxidation was assessed in the following way. Myocardial-membrane phospholipid from the rat heart was isolated, purified, and exposed (as liposomes) to peroxidative-injury stimulus (XOD + HX + Fe^{3+}·ADP) at 37°. Liposome samples were also incubated in parallel, but without XOD + HX + Fe^{3+}·ADP. The net production of lipid conjugated diene (●) and TBA-reactive substance (as MDA-equivalents) (▲) was calculated over a 180-min peroxidation reaction period as the difference in diene and TBA-reactive substance content between the peroxidized samples and their respective non-peroxidized controls. Data points are means ± SD (N = 5).

test substance was taken as evidence of its O_2^- scavenging potential [38]. The assay contained: 0.25 mM potassium phosphate buffer, pH 8.6; 10^{-4} M EDTA; 2 mM NaOH; air-saturated dimethyl sulfoxide (DMSO) containing 0.55 M water; and 76 μM ferricytochrome *c*. The linear rate of SOD-inhibitable cytochrome *c* reduction was monitored at 550 nm, and attenuation of this rate by test substance was considered evidence of the O_2^--trapping ability of the substance.

XOD activity. XOD was assayed spectrophotometrically by monitoring the conversion of xanthine substrate to uric acid at 25° [39]. The assay mixture contained: 50 mM potassium-phosphate buffer, pH 7.8; 10 μM EDTA; 4.2×10^{-9} M catalytically flavin-active XOD; and 0.5 mM xanthine. These conditions have been demonstrated [39, 40] to be optimal with respect to linearity of absorbance rise relative to XOD activity and obviate both interference by iron [40] and accumulation of xanthine if hypoxanthine or purine were used as substrate [41]. Some incubations included an oxygenase inhibitor at concentrations specified in the text.

RESULTS

Kinetics of myocardial phospholipid peroxidation and peroxide decomposition. As detailed [35], myocardial membranes of the rat heart are readily damaged upon exposure to O_2^- and iron-dependent free radical reactions because of membrane phospholipid PUFA peroxidation. Incubation of the purified cardiac-membrane phospholipid (as liposomes) under O_2^--dependent, iron-promoted peroxidation conditions led to a rapid, net formation of lipid conjugated-diene precursors of fatty-peroxides (Fig. 1). Formation of O_2^-, assessed directly as SOD-inhibitable cytochrome *c* reduction, was quasi-linear for ~15 min and was detectable, albeit relatively slight, thereafter. Some 60 nmol O_2^- was produced by 20 min with a maximal initial rate of ~4 nmol O_2^-/min. Since the rate of diene formation during the period of maximal O_2^- production was only slightly greater than the rate when O_2^- production had virtually ceased, the lipid peroxide formation in Fig. 1 mainly reflects propagatory interactions between PUFAs and lipid radicals [42]. By 90 min of reaction, the level of conjugated intermediates in the peroxidation system was maximal, indicating that propagation had ended.

The peroxidation system also developed TBA-reactivity, which, as our laboratory has established [35], largely reflects the MDA end-product of lipid peroxide decomposition under our TBA-test conditions (Fig. 1). A maximal linear rate of BTA-reactive substance (as MDA-equivalents) formation [2.1 ± 0.1 nmol/mEquiv. phospholipid ester reacted/min (mean ± SD, N = 5)] was maintained through the first 45 min of peroxidation to yield some 150 nmol MDA-equivalents by 2 hr.

To study the potential influence of oxygenase inhibitors on myocardial phospholipid peroxidation, a 60-min peroxidation reaction was adopted. As demonstrated by the data in Fig. 1, at 60 min the amplitude of peroxidation was high, and the reaction kinetics remained reasonably linear.

Effects of cardioprotective oxygenase inhibitors on myocardial phospholipid peroxidation. Eight oxygenase inhibitors known to salvage damaged myocardium in occlusion-reperfusion animal models of cardiac injury [10, 12–22] were evaluated for their abilities to inhibit cardiac-membrane phospholipid

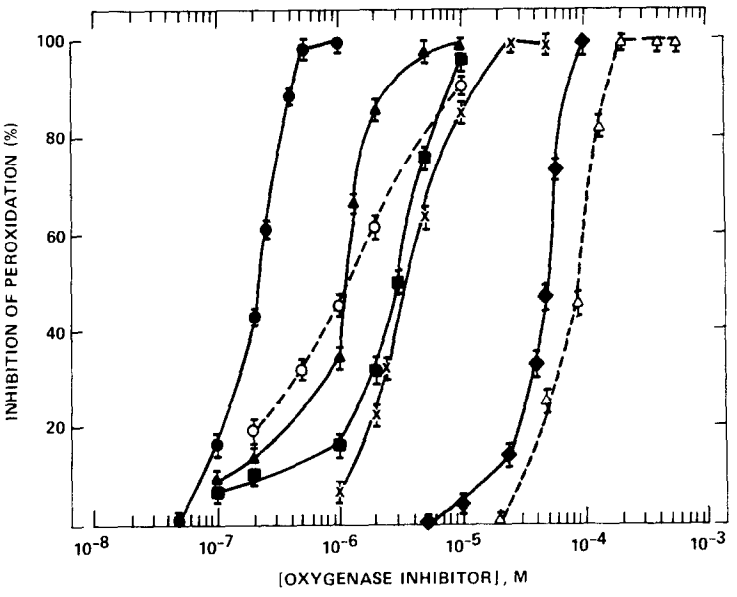

Fig. 2. Inhibition of myocardial phospholipid peroxidation by BW755C (●), AA-861 (▲), catechin (--○--), nafazatrom (■), dipyridamole (×), propyl gallate (♦), and phenidone (--△--). Myocardial membrane phospholipid was exposed to free radical generator (XOD + HX + Fe^{3+}·ADP) for 60 min either in the absence or presence of each listed oxygenase inhibitor at various final concentrations. Inhibition of peroxidation (assessed as TBA-ractivity) is expressed relative to 0% inhibition of the reaction without test substance.Data are means ± SD (N = 4).

peroxidation. The cardioprotective cyclooxygenase inhibitor flurbiprofen and the cardioprotective lipoxygenase inhibitors ibuprofen and REV-5901-5, at concentrations up to 1000 μM, did not affect cardiac phospholipid peroxidation. However, two other cardioprotective lipoxygenase inhibitors, AA-861 and dipyridamole, displayed very effective, concentration-dependent activity against oxidative phospholipid PUFA injury (Fig. 2). AA-861, with an antiperoxidant IC_{50} (concentration at which peroxidation was inhibited by 50%) of 1.25 ± 0.08 μM,was some 3-fold more potent then dipyridamole IC_{50} = 3.6 ± 0.2 μM) (means ± SD, N = 4). AA-861 prevented myocardial phospholipid peroxidative injury at 10 μM, whereas 25 μM dipyridamole was required to block peroxidation.

Three dual oxygenase inhibitors with cardioprotective properties, nafazatrom, propyl gallate, and BW755C, were also effective antiperoxidants. The concentration-dependency of the protective effects of nafazatrom and propyl gallate in our peroxidative injury system (Fig. 2) allowed determination of their respective antiperoxidant IC_{50} values, 3.0 ± 0.2 and 50.0 ± 2.7 μM. Nafazatrom inhibited peroxidation by 95% at 10 μM, whereas propyl gallate blocked peroxidation at a concentration of 100 μM. BW755C was significantly (over 10-fold) more potent an antiperoxidant than the other two dual oxygenase inhibitors tested, having an antiperoxidant IC_{50} of 0.22 ± 0.01 μM. At 1.0 μM, BW755C totally inhibited peroxidation. The antiperoxidant efficacy of BW755C at submicromolar concentrations made this compound the most potent aniperoxidant identified among the cardioprotective oxygenase inhibitors tested.

Antiperoxidant efficacy of non-cardioprotective oxygenase inhibitors. Four cyclooxygenase inhibitors (aspirin, indomethacin, naproxen, and sulfinpyrazone) are ineffective against myocardial inaction in animal models of occlusion-reperfusion injury [23–25]. These agents were found not to protect myocardial membrane phospholipid from peroxidative damage at concentrations up to 500 μM. In fact, aspirin promoted lipid peroxidation in our system, by some 20% at 500 μM.

Antiperoxidant properties of catechin and phenidone. The potential of catechin and phenidone to act as anti-ischemic agents has not been characterized in occlusion-reperfusion cardiac-injury models. Since both are dual oxygenase inhibitors [26, 27], we also assessed whether they could inhibit O_2^--dependent, iron-promoted cardiac phospholipid peroxidation. Catechin and phenidone did display concentration-dependent antiperoxidant activity, with respective antiperoxidant IC_{50} values of 1.4 ± 0.1 and 90 ± 4 μM (Fig. 2). At 10 μM, catechin inhibited peroxidation by 90% whereas a phenidone concentration in excess of 100 μM was required to inhibit peroxidation to a similar degree.

Oxygenase inhibitor-antiperoxidants as O_2^- scavengers and XOD inhibitors. The antiperoxidant efficacy of some oxygenase inhibitors in a XOD-dependent, O_2^--driven, iron-promoted peroxidation system prompted us to test directly whether the oxygenase inhibitor-antiperoxidants identified could inhibit XOD or scavenge O_2^-. Each compound was tested at its antiperoxidant IC_{50} as well as at a concentration that inhibited peroxidation by > 90%. Oxygenase inhibitor-antiperoxidants, at concentrations that significantly reduced or even blocked cardiac phospholipid damage through XOD-dependent, O_2^--driven, iron-promoted oxy-radical chemistry, could neither inhibit XOD [39] nor scavenge O_2^- [38]. In these tests, known XOD inhibitors and

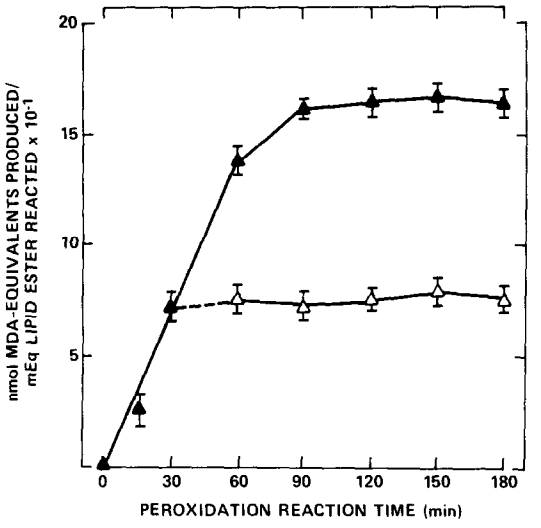

Fig. 3. Effect of BW755C on the progression of peroxidative damage to myocardial phospholipid. Two identical samples of myocardial-membrane phospholipid purified from the rat heart were exposed (as liposomes) to peroxidative stimulus (XOD + HX + Fe^{3+}·ADP) at 37°. Two samples of the liposome suspension alone (i.e. without XOD + HX + Fe^{3+}·ADP) were also incubated in parallel. At 30 min, BW755C (10 μM, final conc.) (△) or the equivalent volume of buffer (▲) was added to one of the two respective, on-going peroxidation reactions and to one of the two respective liposome suspensions. The net production of TBA-reactive substance (as MDA-equivalents) was calculated over a 180-min peroxidation reaction period as the difference in the content of TBA-reactive substance between each peroxidized sample and its respective, non-peroxidized control. Data points are the means of four determinations ± SD.

O_2^- scavengers were effective at (or below) a low micromolar concentration: for example, 8 μM allopurinol inhibited XOD by some 50%, and 10 nM SOD scavenged all of the O_2^- produced [40].

Oxygenase inhibitor-antiperoxidants as "chain-breaking" molecules. The ability of oxygenase inhibitor-antiperoxidants to prevent the enzymatic formation of lipid peroxides [2, 6] and their lack of XOD-inhibitory and O_2^--scavenging properties at effective antiperoxidant concentrations (above) suggested that these agents could act mechanistically to interrupt the propagatory, chain-reaction phase of auto-oxidative lipid damage [42]. To investigate this point, each of the various oxygenase inhibitor-antiperoxidants was introduced at 30 min into an on-going O_2^--, iron-dependent peroxidation reaction (Fig. 1) at a concentration known to prevent fatty-peroxide formation if present at the start of the reaction (Fig. 2). This time was selected from consideration of the data in Fig. 1 which demonstrate that, by 30 min, peroxyl radical-PUFA interactions were actively amplifying the O_2^--dependent initiation, and O_2^- generation from HX + XOD was negligible.

As exemplified by BW775C (Fig. 3), the oxygenase inhibitor-antiperoxidants identified could prevent peroxidative lipid injury by interrupting the propagatory chain-reaction between fatty-peroxyl radicals and PUFAs. This "chain-breaking" mode of action not only brought about a halt in the on-going formation of lipid peroxides under peroxidant stress, but also reduced the extent of peroxidative damage and limited the injury over time (Fig. 3).

DISCUSSION

Eicosanoids can have deleterious actions on coronary circulation and myocardial function [4]. Consequently, oxygenase inhibitors are being evaluated pharmacologically as potential anti-ischemic therapeutics [10]. Some, but not all, oxygenase inhibitors limit infarction in experimental models of ischemic cardiac injury [10] and suppress leukocyte accumulation in the ischemic myocardium [18]. The rapid, reversible nature of the oxygenase inhibition displayed by known antioxidants and some cardioprotectants has focused interest on the potential antioxidant properties which oxygenase inhibitors may possess and which may be critical to myocardial salvage through oxygenase inhibition [2, 5, 6]. Although non-oxygenase mediated myocardial-membrane phospholipid peroxidation may be an important pathogenic factor in ischemic myocardial necrosis [8, 28, 29], quantitative study of the antiperoxidant properties of cardioprotective oxygenase inhibitors in a pathophysiologically relevant oxidative-injury system is lacking. Recent reviews [2, 3, 6, 10] offer the opinion that additional biochemical information is needed to define and characterize the *in vivo* physiologic and pharmacologic effects of oxygenase inhibitors.

The results of this investigation provide compelling evidence that some cardioprotective oxygenase inhibitors possess appreciable antioxidant activity as "chain-breaking" molecules and can thereby prevent peroxidative injury to myocardial-membrane phospholipid PUFAs. The cardioprotective oxygenase inhibitor-antiperoxidants identified display antiperoxidant IC_{50} values over an approximately 200-fold range. The extremes are represented by two dual inhibitors, BW755C (IC_{50} = 0.22 μM) and propyl gallate (IC_{50} = 50 μM), although phenidone, whose anti-ischemic efficacy is not known, was ~2-fold less potent an antiperoxidant than propyl gallate. The absolute potencies of these agents as oxygenase inhibitors vary with, for example, assay conditions, enzyme preparations, and cell/tissue enzyme source [43]. Yet the cardioprotectant oxygenase inhibitor-antiperoxidants, BW755C, AA-861, nafazatrom, dipyridamole, and propyl gallate, as well as catechin and phenidone, are oxygenase enzyme inhibitors at low micromolar concentrations approximating their concentrations as antiperoxidants effective against O_2^--dependent, iron-promoted membrane lipid injury [5, 6, 43, 44]. On the other hand, oxygenase inhibitors without detectable antiperoxidant activity at a 1.0 mM concentration (e.g. flurbiprofen, ibuprofen, aspirin) act as enzyme inhibitors at micromolar or even submicromolar concentrations [5, 6, 43]. Thus, specific correlations between the relative potencies of these compounds as oxygenase inhibitors and as antiperoxidants are neither mechanistically informative nor predictive, but could be suggestive of an adverse antioxidant influence on enzymatic PUFA oxy-

genation. Accordingly, the fact that aspirin is not an effective antiperoxidant implies that cyclooxygenase inhibition by aspirin is not radical-dependent, but does not even hint at the requirement for irreversible acetylation of the active site of the enzyme in this inhibition [45]. Likewise, further experimentation would be required to define the disposition and myocardial tissue levels of these compounds in order to correlate antiperoxidant potency, oxygenase inhibition, and cardioprotection in various animal models of ischemic heart disease.

Our findings that five anti-ischemic oxygenase inhibitors also prevented non-enzymatic, radical-dependent cardiac phospholipid peroxidation at concentrations inhibitory to oxygenases would support the possibility that these agents exert cardioprotective effects by directly influencing the sensitivity of myocardial membrane phospholipid to free radical attack. The potential pharmacologic significance of this antiperoxidant activity against non-enzymatic lipid peroxidation is enhanced by recent demonstrations of an intimate temporal association between O_2^--driven, iron-promoted phospholipid peroxidation and heart-muscle injury during cardiac ischemia [31] and reperfusion [32], with XOD a potential O_2^- source [30]. The antiperoxidative efficacy of some anti-ischemic oxygenase inhibitors could also prevent neutrophil-derived radicals and eicosanoids from damaging the heart-muscle cell [10]. Such anti-inflammatory activity against leukocytes has been described for the cardioprotective oxygenase inhibitor-antiperoxidant BW755C [46].

No oxygenase inhibitor-antiperoxidant tested could directly scavenge O_2^- or inhibit XOD at concentrations which prevented O_2^--(XOD-) dependent, iron-promoted peroxidation. The great qualitative and quantitative differences between the antiperoxidant profiles of oxygenase inhibitors (Fig. 2) and iron chelators [35, 40] in our oxidative injury system make it unlikely that the antiperoxidant effect of some oxygenase inhibitors is due to their removing iron from the system through chelation. Conversely, (poly)phenolic, "chain-breaking" antioxidants, including vitamin E and BHT, have antiperoxidant efficacies at low micromolar concentrations reminiscent of the oxygenase inhibitor-antiperoxidants studied here [35, 40]. Papatheofanis and Lands [44] have hypothesized, in fact, that the ability of propyl gallate to act as a rapid, reversible lipoxygenase inhibitor is due to its direct antagonism of the enzymatically-controlled, free-radical lipid chain reaction. The data in Fig. 3 are in accord with this thinking and indicate that the antiperoxidant activity of some oxygenase inhibitors appears to be as a result of their ability to terminate the propagation of lipid peroxidation by preventing the interaction of lipid radicals with PUFAs [47].

Duniec *et al.* [48] have reported on the effects of nine compounds as cyclooxygenase inhibitors and as inhibitors of ascorbic acid-dependent, rat-liver microsome peroxidation. Our data in a different peroxidation system support their results that aspirin and indomethacin have no antiperoxidant properties, whereas BW755C has. In our hands, however, 500 μM aspirin stimulated peroxidative lipid injury, and a recent report [49] suggests that enhancement of lipid peroxidation is a mechanism whereby aspirin induces liver damage. The data of Duniec *et al.* [48] characterizing the antiperoxidant properties of the cyclooxygenase inhibitors KD 785, phenylhydrazine, caffeic acid, chlorpromazine, eicosatetraynoic acid, and paracetamol have been extended by our present work to include the antiperoxidant properties of the oxygenase inhibitors flurbiprofen, AA-861, dipyridamole, ibuprofen, REV-5901-5, nafazatrom, propyl gallate, naproxen, sulfinpyrazone, catechin, and phenidone. It is worthy of note that, of all the oxygenase inhibitors tested in the two peroxidation systems, BW755C was the most potent antiperoxidant, effective at submicromolar concentrations. The antiperoxidant properties of BW755C and of the other cardioprotective oxygenase inhibitors identified herein should be borne in mind when dissecting pharmacologic responses to these agents.

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