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PROTECTION OF RAT MYOCARDIAL PHOSPHOLIPID AGAINST PEROXIDATIVE INJURY THROUGH SUPEROXIDE- (XANTHINE OXIDASE)-DEPENDENT, IRON-PROMOTED FENTON CHEMISTRY BY THE MALE CONTRACEPTIVE GOSSYPOL

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Abstract—Metal-promoted oxygen free-radical chemistry is a cause of tissue damage in many disease states, such as myocardial ischemia. The effect of gossypol, a polyphenolic plant pigment and male contraceptive, on the peroxidation of myocardial membrane phospholipid was studied and quantitatively characterized. As a result of exposure to xanthine oxidase (superoxide)-dependent, iron-promoted Fenton chemistry, cardiac phospholipid was readily peroxidized with defined kinetics. The peroxidation could be blocked by substances which interdict at specific points in the Fenton chemistry: superoxide dismutase, α-tocopherol, the iron chelator desferrioxamine, and the xanthine oxidase substrate-analogs allopurinol and oxypurinol. The oxidative-injury system displayed a characteristic antiperoxidant response to each type of inhibitor. Gossypol, at low micromolar concentrations, profoundly altered the rate and extent of myocardial phospholipid peroxidation. Gossypol was ineffective as a xanthine oxidase inhibitor and as a superoxide scavenger at concentrations that abolished myocardial lipid peroxidation. Since metal chelation was an effective means of preventing lipid peroxidation in this system only when the iron therein was completely chelated, the low anti-peroxidant IC_{50} for gossypol, 1.1. μM, relative to the concentration of iron (100 μM) did not support a functionally significant antiperoxidant role for gossypol as an iron chelator. Rather, it appears that, at low micromolar gossypol concentrations which approximate the peak plasma concentrations in humans, the antiperoxidant effects of gossypol against superoxide-mediated, iron-promoted lipid damage rest with the ability of gossypol to intercept lipid radical intermediates as a "chain-breaking" aromatic phenol.

Gossypol [(2,2′-binaphthalene)-8,8′-dicarboxaldehyde-1, 1′, 6, 6′, 7, 7′-hexahydroxy-5, 5′-diisopropyl-3,3′-dimethyl; see Fig. 4, *inset*], a yellow polyphenolic pigment from cottonseed oil, has been studied for its potential pharmacologic value as an anticancer [1], antiviral [2], and antiallergy [3] agent and in the treatment of endometriosis [4] and trypanosomal infections [5]. The compound has a variety of cellular effects as an enzyme inhibitor of, for example, adenylate cyclase [6] and arachidonate 5- and 12-lipoxygenases [7]. Perhaps the most extensive information on the action of gossypol concerns its antispermatogenic and spermicidal effects [8] and its clinical efficacy as a male contraceptive [9]. Although its mode of action even as a contraceptive is not well understood, gossypol is highly lipophilic and can interact directly with the phospholipid bilayer of biological membranes to alter membrane molecular packing [10], electrostatic charge [11], and transmembrane ion fluxes [12], with consequent functional changes in membraneous organelles [13].

Gossypol, in common with many other aromatic phenols, is an antioxidant [14]. Despite its long-standing use in the rubber and fats-and-oils industries [14], gossypol's biologic activity in preventing the peroxidation of membrane (phospholipid) polyunsaturated fatty acids has received little study. Whereas some workers have demonstrated that gossypol can inhibit the ascorbate-induced peroxidation of rat liver microsomes [15] and human spermatozoa [16], other studies have indicated that gossypol promotes the formation of highly-reactive oxygen-containing free radicals, particularly $O_2^{\cdot-}$,† the superoxide anion radical [17].

An increasing body of experimental evidence has implicated membrane peroxidation mediated by $O_2^{\cdot-}$-driven, metal-promoted Fenton chemistry [18, 19] in the pathogenesis of tissue injury, such as ischemic and reperfusion heart damage [20, 21]. Specifically, key roles have been assigned to xanthine oxidase (XOD; xanthine:oxygen oxidoreductase,

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† Abbreviations: $O_2^{\cdot-}$, superoxide anion radical; XOD, xanthine oxidase; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; NDGA, nordihydroguaiaretic acid; HTP, 4-hydroxymethyl-2,6-di-terbutyl phenol; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; ADP, adenosine diphosphate; TBA, 2-thiobarbituric acid; NBT, nitro-blue tetrazolium; SOD, superoxide dismutase; TCA, trichloroacetic acid; DMSO, dimethyl sulfoxide; and MDA, malondialdehyde.

EC 1.2.3.2.) as (enzymatic) generator of $O_2^{\cdot-}$ and to iron (Fe^{2+}/Fe^{3+}) as tissue redox couple in the formation of the fatty-acyl lipid radicals being identified and spin-trapped in heart muscle during myocardial ischemia [22, 23]. Pharmacokinetic and metabolic studies in the male rat [24, 25] have consistently identified the heart as a principal site of gossypol deposition. These considerations, along with the reported efficacy of gossypol not only as an antioxidant, but also as an iron chelator [15, 16], an XOD inhibitor [3], and a lipid peroxidant [17], prompted us to study, quantitatively, the effects of gossypol in a cardiac phospholipid peroxidation system supported by XOD-dependent, iron-promoted radical chemistry. The influence of gossypol on lipid peroxidation will be placed in the comparative context of the effects of other (poly)phenolics [butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), α-tocopherol, propyl gallate, quercetin, nordihydroguaiaretic acid (NDGA), and 4-hydroxymethyl-2,6-di-*ter*butyl phenol (HTP)] [27–30], iron chelators (desferrioxamine) [31], and XOD inhibitors (allopurinol and oxypurinol) [32].

MATERIALS AND METHODS

Materials. Gossypol, BHA, BHT, propyl gallate, NDGA, allopurinol, oxypurinol, hypoxanthine, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), tris(hydroxymethyl)aminomethane (Tris), adenosine diphosphate (ADP), 2-thiobarbituric acid (TBA), tetramethoxypropane, nitro-blue tetrazolium (NBT), and hydroxylamine hydrochloride were from Sigma (St. Louis, MO, U.S.A.). HTP was purchased from the PolyScience Corp. (Niles, IL, U.S.A.). 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 cytochrome *c* (from horse heart) were purchased from Boehringer-Mannheim (Indianapolis, IN, U.S.A.). Desferrioxamine B methanesulfonate (Desferal) was a gift from Ciba A.G. (Basle, Switzerland). Ferrioxamine was synthesized according to Ref. 31. α-Tocopherol was synthesized by Hoffmann-La Roche Inc. All solvents were of analytical grade as purchased (Burdick & Jackson, Muskegon, MI, U.S.A.).

Isolation and purification of rat myocardial lipids. The procedure was carried out in a dehumidified cold-room (3°). Conscious male, Sprague–Dawley rats (~275 g; Charles River, Boston, MA, U.S.A.) fed a normal rodent diet *ad lib.* were decapitated. The hearts were rapidly excised and perfused via the aorta with 20.0 ml of ice-cold 10 mM HEPES buffer, pH 7.4. The aorta and atria were removed, and the trimmed ventricular myocardium was minced and homogenized (100 mg tissue/ml of ice-cold HEPES) for 3 × 5 sec with a Tissuemizer (Tekmar, Cincinnati, OH, U.S.A.) at "maximal" setting. The homogenate was filtered through four-ply cheesecloth, and the myocardial lipids were extracted and purified from the filtrate by a modified Bligh–Dyer [33] procedure as described [34]. The final chloroform phase containing the purified lipid represents quantitative recovery of myocardial lipid: >98% of the radioactivity associated with the organic fraction obtained by direct alkaline saponification [35] of myocardium metabolically labeled from [^{14}C]acetate, sodium salt (New England Nuclear, Boston, MA, U.S.A.), was recovered in the saponified fraction from the myocardial lipid extracted out of an equivalent amount of tissue. The total myocardial lipid was resolved into its constituent phospholipid and neutral lipid fractions by silica column chromatography on Sep-Pak cartridges (Waters, Milford, MA, U.S.A.) [36]. All lipids were stored in chloroform under nitrogen at −20° [37].

Lipid quantification. Lipid phosphate was determined microchemically [38] on perchloric acid digests using KH_2PO_4 as standard.

The hydroxamate reaction [39] was used to measure lipid ester. Aliquots of lipid extract were evaporated to dryness under nitrogen. One-half milliliter of ethanol:methyl-*t*-butyl ether (3:1, v/v) was added to solubilize the dried extract, followed by 0.2 ml of a 1:1 (v/v) mixture of 2.0 M hydroxylamine hydrochloride and 3.5 M NaOH. The tubes were agitated and sealed, and after 20 min at room temperature 0.1 ml of 5.0 N HCl and 0.1 ml of 0.37 M $FeCl_3$ in 0.1 N HCl were added. The absorbance of the fatty-acyl hydroxamic acid derivatives was read at 520 nm. L-α-Phosphatidylcholine dipalmitate (Avanti Polar Lipids, Birmingham, AL, U.S.A.) was used as standard.

Preparation of cardiac liposomes. A known amount of cardiac lipid in chloroform was placed in a glass flask and evaporated to dryness under nitrogen at room temperature; the flask was rotated gently during evaporation to yield a thin, dry lipid film. The lipid was taken up in 10 mM HEPES–0.145 M KCl, pH 7.4, and was resuspended by indirect anaerobic sonication for 15 min at room temperature. The liposome suspension was used immediately.

Peroxidation reaction system. Cardiac liposomes were subjected to $O_2^{\cdot-}$-dependent, iron-promoted peroxidation in glass test tubes to avoid the antioxidant effects of polymerizing agents commonly used in fabricating plastic labware [40]. Experiments involving α-tocopherol were conducted under amber light. Ninety minutes before the start of the reaction, a chelate was formed in 10 mM HEPES–0.145 M KCl buffer, pH 7.4, between Fe^{3+} (1.0 mM $FeCl_3$) and 10 mM ADP with continuous stirring at room temperature; the chelate was freshly prepared for each experiment to ensure solubility and effective chelation. The peroxidation reaction, in a final volume of 1.0 ml, contained the following components at their specified final concentrations: 10 mM HEPES–0.145 M KCl, pH 7.4; 1.0 mM hypoxanthine; 0.1 mM Fe^{3+}–1.0 mM ADP chelate; 125 μg lipid (as liposomal suspension); and 10 mUnits XOD. The reaction was started upon adding the XOD, mixing, and incubating at 37° in a shaking water bath. Peroxidation was terminated by adding 0.15 ml of 76% (w/v) in trichloracetic acid (TCA) in 2.3 N HCl and immersing the sample in an ice-water bath. Concentrated stock solutions of gossypol, quercetin, BHA, BHT, HTP and NDGA were made in dimethyl sulfoxide (DMSO); α-tocopherol and propyl gallate were solubilized in ethanol; des-

ferrioxamine, ferrioxamine, allopurinol, and oxypurinol were solubilzed in the HEPES–KCl buffer. Gossypol concentrations were verified spectrophotometrically [41]. The final concentration of DMSO or ethanol in the peroxidation reaction never exceeded 0.005 vol%. a concentration which did not influence cardiac lipid peroxidation and which was well (~1000-fold) below the concentration where an effect could be noted.

Determination of lipid peroxidation. Malondialdehyde (MDA)-equivalents were measured as TBA-reactive material by the following modification of published methods [42, 43]. The reaction mixture, prepared daily, contained water:BHT (7.145 M BHT in absolute ethanol):TBA (1.514 wt% TBA in 0.2 M Tris, pH 7.0) in the volume ratio 1:1:5. To each 1.15 ml of acidified peroxidation reaction (above), 0.35-ml reaction mixture was added. Upon thorough mixing, the samples were incubated in an 80° shaking water bath for 30 min. After this time, the tubes were plunged into an ice-water bath, and the TBA test was stopped with 0.5 ml of ice-cold 90% (w/v) TCA followed by 2.0 ml $CHCl_3$. After centrifugation for 30 min at 2000 *g* (4°), the absorbance of the upper phase was read at 532 nm. A standard curve (0.8 to 40.0 nmol MDA) was run with every assay, for which MDA was prepared by acidification of 1,1,3,3-tetramethoxypropane with 76% (w/v) TCA in 2.3 N HCl. Computer-assisted regression analysis of the standard curve was used to quantify the molar amounts of MDA-equivalents in the experimental samples. Under these conditions, none of the studied compounds influenced color development from MDA standard and evidenced no TBA-reactivity themselves. Dose–response curves were generated with the assistance of RS/1 software (BBN Corp. Cambridge, MA, U.S.A.) on an IBM PC-AT (IBM Corp., Boca Raton, FL, U.S.A).

Conjugated diene intermediates of lipid peroxidation were measured spectrophotometrically [44].

XOD assay. XOD was assayed by monitoring the conversion of xanthine to uric acid with a Beckman DU-7 kinetic spectrophotometer (Beckman Instruments, Palo Alto, CA, U.S.A.) 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 determined [45] to be optimal with respect to substrate concentration and linearity of absorbance rise relative to XOD activity and obviate both interruption of the reaction at the level of xanthine if hypoxanthine or purine were used as substrate [46] and interference by Fe^{3+} [47].

Assessment of O_2^- scavenging. The O_2^- generated from hypoxanthine + XOD was reacted with either cytochrome *c* or NBT as detector molecule. The reaction mixture, in a final volume of 3 ml, consisted of: 76 μM cytochrome *c* or 100 μM NBT, 1.0 mM hypoxanthine, 10 mUnits XOD, and 50 mM potassium-phosphate buffer, pH 7.4, containing 0.1 mM EDTA. The linear rate of SOD-inhibitable cytochrome *c* reduction was monitored at 550 nm [48]; the linear rate of SOD-inhibitable NBT reduction was monitored at 560 nm [49]. Gossypol was tested for its ability to prevent the SOD-inhibitable reduction of both detector molecules as indication of its O_2^--scavenging capability.

Protein assay. Tissue protein was quantified with a dye-binding technique [50].

RESULTS

The total myocardial lipid complement of the rat heart ventricular muscle mass was readily peroxidized upon exposure to O_2^--dependent, iron-promoted Fenton chemistry with hypoxanthine + XOD + Fe^{3+}-ADP as free radical generator (Fig. 1). Conjugated diene intermediates of lipid peroxidation were produced within 10.0 min, accompanied by rapid lipid (endo)peroxide breakdown to TBA-reactive substance, which we have demonstrated elsewhere [51] to be MDA. Increasing linear accumulation of both dienes and MDA defined a propagation phase, during which a maximal peroxidation rate of 3.0 ± 0.2 nmol MDA-equivalents produced/mEq lipid ester reacted/min was attained. By 45 min of reaction, the net production of conjugated diene from total myocardial lipid declined with the progressive conversion of conjugated structures into lipid peroxides and breakdown products, including MDA. The attenuation of peroxidation rate occurred as the non-peroxidized polyunsaturated fatty-acyl content of the system became limiting. The production of O_2^-, measured directly with cytochrome *c* or NBT as detector molecule, was linear for over 70 min and was still taking place at 120 min; no further lipid peroxidation was observed by supplementing the system with additional free radical generator (data not shown). As indicated by the greatly reduced development of TBA-reactivity after 90 min, myocardial lipid peroxidation was essentially complete within 2 hr.

Cellular membranes are the principal sites of free radical-induced tissue damage, for they contain in their fluid bilayers virtually all of the tissue phospholipid [52]. Consequently, we studied the peroxidation of myocardial phospholipid and neutral lipid as separated out of the total myocardial lipid extract (Fig. 1). The myocardial neutral lipid, which represented ~19% of the total myocardial lipid complement, did not display a significant peroxidative response during incubation with hypoxanthine + XOD + Fe^{3+}-ADP. The myocardial phospholipid, however, was peroxidized without a detectable lag period to attain maximal, linear peroxidation rates in the propagation phase equal to the maximal rates of total cardiac lipid peroxidation. This shift of the peroxidation progress curve to the left when neutral lipid was eliminated from the system reflects removal thereby of tissue α-tocopherol [53]. The net formation of TBA-reactive substance during myocardial phospholipid peroxidation was equal to that formed from peroxidation of the total myocardial lipid. These results demonstrate that myocardial membrane phospholipid, whether purified out of the total myocardial lipid extract or not, was the substrate for free radical attack.

The antiperoxidant efficacies of compounds having the ability to interdict at critical points in XOD (O_2^-)-dependent, iron-promoted Fenton chemistry were quantitatively determined (Fig. 2). SOD,

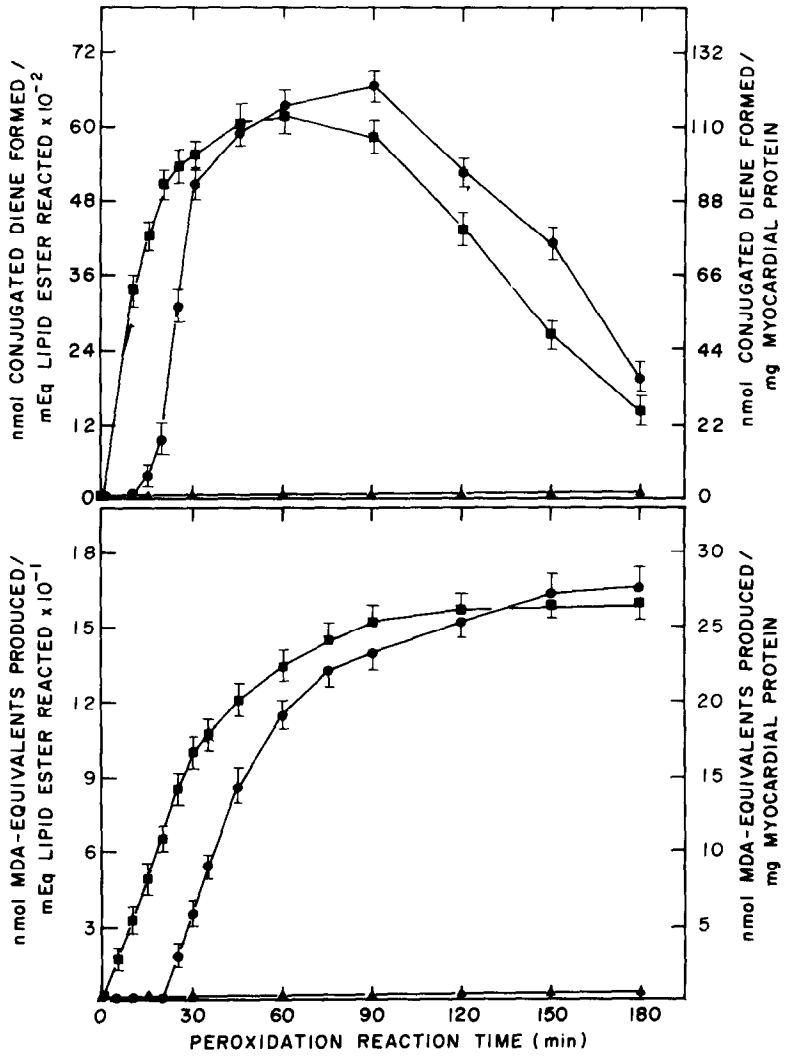

Fig. 1. Time-course of cardiac lipid peroxidation through $O_2^{\cdot-}$-dependent, iron-promoted Fenton chemistry. Total lipid (●) and the phospholipid (■) and neutral lipid (▲) subfractions therefrom were isolated and purified from rat myocardium and exposed, as liposomal suspension, to free radicals generated by XOD + hypoxanthine + Fe^{3+}-ADP. Resultant lipid peroxidation was quantified both as production of conjugated intermediates (top panel) and as TBA-reactivity (bottom panel). Data points are means ± SD ($N \geq 5$).

which specifically dismutates $O_2^{\cdot-}$ ($2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$) and thereby removes from the system a radical substrate essential to Fenton chemistry [18, 19], prevented myocardial phospholipid peroxidation at low nanomolar concentrations, with an antiperoxidant IC_{50} of 3.8 ± 0.2 nM. α-Tocopherol, a chain-breaking antioxidant which intercepts carbon-centered lipid radicals [54], displayed an antiperoxidant IC_{50} of 0.8 ± 0.1 μM and virtually blocked myocardial phospholipid peroxidation at 10.0 μM. Desferrioxamine, a stoichiometric iron chelator used clinically as a therapeutic against iron overload [31], prevented peroxidation only at concentrations at least as great as the iron concentration in the system (100 μM), suggesting that iron chelation was an effective means of antiperoxidant protection only when iron substrate for the Fenton reaction had been totally chelated and thereby functionally removed from the peroxidation system. This conclusion is supported by the fact that ferrioxamine at ≥100 μM did not inhibit peroxidation, while its rate constant for reaction with hydroxyl radical is the same as that of desferrioxamine [31]. Allopurinol and oxypurinol, substrate (purine) analog-inhibitors of XOD but not $O_2^{\cdot-}$ (or hydroxyl radical) scavengers [32], acted as antiperoxidants at high micromolar concentrations which approached the concentration of XOD substrate (1.0 mM) in the reaction.

Gossypol, even at low micromolar concentrations, had profound effects upon the magnitude and kinetics of myocardial phospholipid peroxidation by the SOD-dependent, iron-promoted radical generator (Fig. 3). Gossypol delayed the initiation of per-

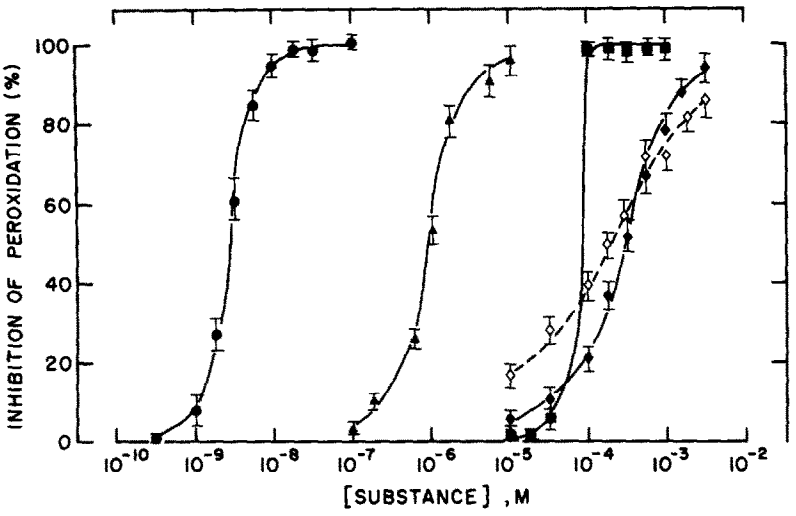

Fig. 2. Inhibition of cardiac lipid peroxidation by SOD (●), α-tocopherol (▲), desferrioxamine (■), oxypurinol (◇), and allopurinol (◆). Myocardial lipids were exposed to free radical generator (XOD + hypoxanthine + Fe^{3+}-ADP) for 60 min either in the absence of presence of each listed substance at various final concentrations. Inhibition of peroxidation (assessed as TBA-reactivity) is expressed relative to zero percent inhibition for the reaction without test substance, and data are given as means ± SD ($N \geq 5$).

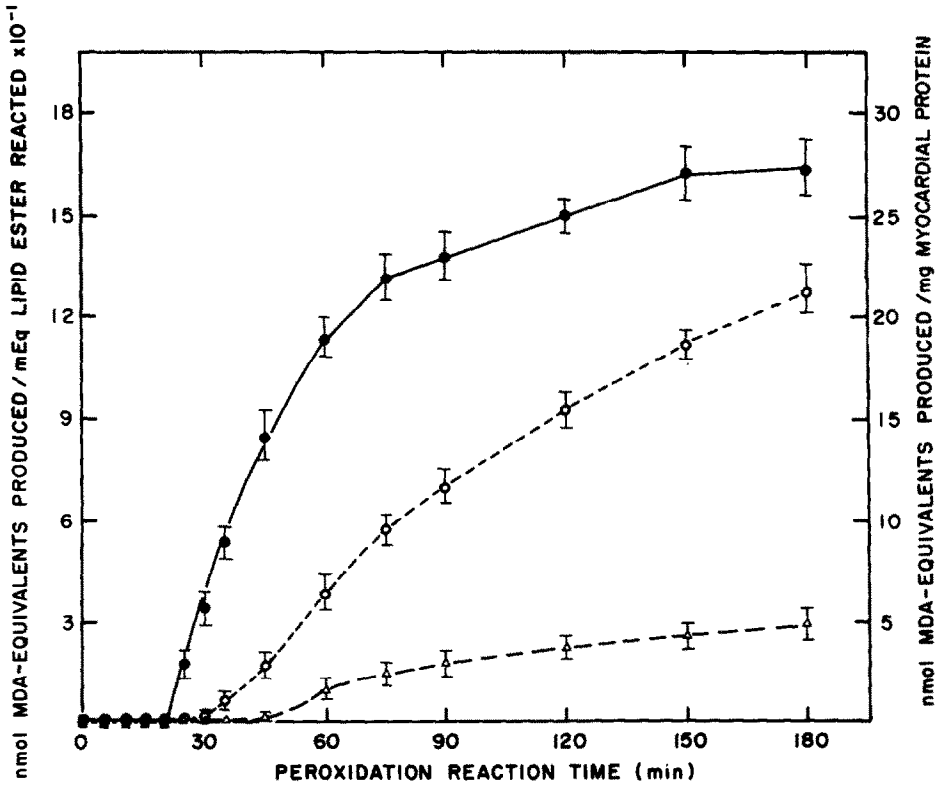

Fig. 3. Effect of gossypol on the kinetics of myocardial lipid peroxidation. Myocardial lipids were peroxidized through $O_2^{\cdot-}$-dependent, iron-promoted Fenton chemistry with XOD + hypoxanthine + Fe^{3+}-ADP as free radical generator either in the absence (●) or presence (○, 1.0 μM final concn; △, 3.0 μM final concn) of gossypol. Peroxidation was assessed as TBA-reactivity, and values are given as means ± SD ($N \geq 5$).

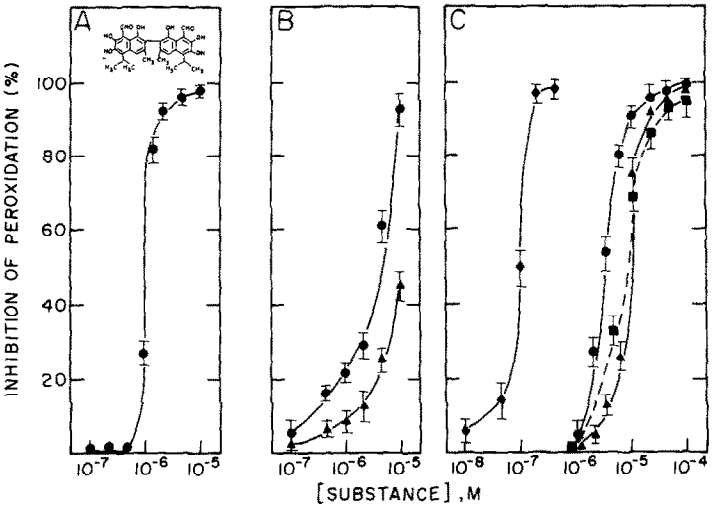

Fig. 4. Inhibition of cardiac lipid peroxidation by gossypol (panel A; structure, *inset*); quercetin (●) and propyl gallate (▲) (panel B); and NDGA (◆), BHA (●), BHT (▲) and HTP (■) (panel C). Myocardial lipids were exposed to free radical generator (XOD + hypoxanthine + Fe^{3+}-ADP) for 60 min either in the absence or presence of each listed substance at various final concentrations. Inhibition of peroxidation (assessed as TBA-reactivity) is expressed relative to zero percent inhibition for the reaction without test substance, and values are given as means ± SD ($N \geq 5$).

oxidation, suppressed the rate of propagation, and greatly inhibited the extent of radical-induced oxidative lipid damage. Direct analysis of the ability of gossypol to scavenge $O_2^{\cdot-}$ demonstrated that gossypol was unable to do so at concentrations which greatly attenuated or even prevented peroxidation.

The concentration-dependence of gossypol's antiperoxidant action was characterized and quantitatively compared to the antiperoxidant behavior of a variety of (poly)phenolic substances (Fig. 4). Gossypol displayed an antiperoxidant IC_{50} of 1.1 ± 0.1 μM against $O_2^{\cdot-}$-dependent, iron-promoted cardiac phospholipid peroxidation. Quercetin, a plant flavonol with antioxidant properties [29], had an antiperoxidant IC_{50} of 8.0 ± 0.4 μM. Propyl gallate and NDGA, chain-breaking antioxidants and lipoxygenase inhibitors [55, 56], protected cardiac phospholipids against oxyradical damage. Although solubility limits prevented precise determination of propyl gallate's antiperoxidant IC_{50}, that of NDGA was 0.1 ± 0.05 μM. BHA, BHT and HTP, widely used to prevent food rancidity [57], displayed antiperoxidant IC_{50} values between 1.0 and 10.0 μM.

The reported ability of low micromolar concentrations of gossypol to inhibit XOD [3] and gossypol's antiperoxidant efficacy in an XOD-dependent, iron-promoted oxidative injury system (Figs. 3 and 4) prompted us to test directly and comparatively the effects of gossypol, allopurinol and oxypurinol on XOD activity under optimal XOD assay conditions [45]. The data from these experiments (Fig. 5) demonstrated that levels of gossypol which prevented cardiac phospholipid peroxidation through XOD-dependent, iron-promoted radical chemistry did not inhibit XOD, whereas concentrations of allopurinol and oxypurinol ineffective as antiperoxidants inhibited XOD by >50%. At no concentration tested (up to 1.0 mM) did gossypol inhibit XOD.

DISCUSSION

For a variety of tissues, the pathophysiological importance of oxidative injury mediated by $O_2^{\cdot-}$ in the presence of transition metals is widely appreciated [18, 19]. In particular, myocardial membrane lipid peroxidation by $O_2^{\cdot-}$-dependent, iron-promoted Fenton chemistry appears to be a critical motive force in the progression of reversible ischemic injury to irreversible infarction [20] and in the acceleration of myocardial reperfusion damage [21]. Although other (poly)phenolics have been studied extensively in experimental models of oxy-radical damage (e.g. [58]), the antiperoxidant effects of gossypol in biological systems of oxidative injury have received very limited quantitative study. Gossypol is known to

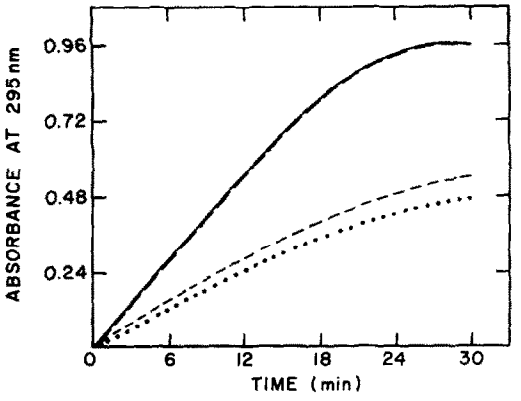

Fig. 5. Effects of gossypol (——), allopurinol (– – –), and oxypurinol (· · ·) on XOD activity. The conversion of xanthine to uric acid by XOD was monitored spectrophotometrically under optimal conditions for XOD activity [45] in the absence (—) or presence (5.0 μM final concn) of each test substance. The graph represents a composite of the kinetic traces of the reaction under each condition.

inhibit the peroxidation of human spermatozoa [16] and rat liver endoplasmic reticulum lipids [15]. Although in both these studies the free radical generator was ascorbate-iron, in the former instance gossypol's antiperoxidant action was attributed to the ability of the molecule to intercept lipid radicals and thereby halt the propagation of peroxidation, whereas in the latter study it was concluded that gossypol inhibited microsomal peroxidation by chelating iron. Furthermore, both these conclusions run counter to the findings of another study [17] which indicate that gossypol, when incubated with human sperm or rat liver microsomes, promotes $O_2^{\cdot-}$ formation and thus causes oxidative tissue injury.

Our experiments have probed the antiperoxidant effects of gossypol within the context of a more complex, yet chemically defined, oxidative injury model that utilizes an enzymatic generator of $O_2^{\cdot-}$, XOD, which is alleged to be inhibited by gossypol [3], along with iron, at physiological pH. The data presented demonstrate that gossypol can effectively prevent the $O_2^{\cdot-}$ (XOD), dependent, iron-promoted peroxidation of myocardial membrane phospholipid and can profoundly attenuate the kinetics and extent of peroxidation. Direct comparison of the antiperoxidant efficacy of gossypol in this system with that of known (poly)phenolic antioxidants, the iron chelator desferrioxamine, and XOD inhibitors offers compelling evidence that gossypol, like some other aromatic, chain-breaking polyphenols, can prevent biological lipid peroxidation through $O_2^{\cdot-}$-dependent, iron-promoted Fenton chemistry by scavenging lipid radical intermediates and not by inhibiting the enzymatic $O_2^{\cdot-}$ generator, XOD, or by removing the iron from the system through chelation. Further supporting evidence for this conclusion was provided by the inability of gossypol, at concentrations which blocked peroxidation, to suppress XOD activity and to scavenge $O_2^{\cdot-}$.

At no concentration of gossypol tested (up to its solubility limit) was Fenton chemistry enhanced or stimulated to promote cardiac lipid peroxidation. It appears likely, therefore, that a metabolic product of gossypol (such as a redox-cycling quinone) may be responsible for the ability of gossypol to generate oxygen radicals and, hence, for some of the side-effects associated with gossypol treatment [9]. Another possible source of gossypol's side-effects, which underscores the importance of studying the actions of this substance in oxy-radical systems, stems from its influence on two key enzymes involved in the oxidative, radical-mediated metabolism of arachidonic acid, lipoxygenase [7] and prostaglandin synthetase [8].

Since the peak concentration of gossypol in the plasma of the human male following a single 20.0 mg dose (p.o.) is ~2.0 μM with a half-life of 10 days [59], it is possible that the usual chronic administration of gossypol as an antifertility agent could afford both contraceptive and antiperoxidant protection. However, in view of its cardiovascular side-effects (including a direct negative inotropic effect and promotion of arrhythmia [12]), it is unlikely that gossypol, although abundant, inexpensive and (even at 5-fold the ordinary antifertility doses) nontoxic, could be used clinically as a membrane-protective antiperoxidant without extensive, quantitative characterization of both its actions in radical-mediated injury systems and its general pharmacology.

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