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INTERACTION OF THE DI-CATECHOLS ROOPEROL AND NORDIHYDROGUAIARETIC ACID WITH OXIDATIVE SYSTEMS IN THE HUMAN BLOOD

A STRUCTURE–ACTIVITY RELATIONSHIP

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Abstract—The effects of the di-catechols rooperol [(*E*)-1,5-bis(3′,4′-dihydroxyphenyl)pent-4-en-1-yne; P2] and nordihydroguaiaretic acid (NDGA) on oxidative systems in the human blood were investigated. P2 and NDGA gave comparable results in the inhibition of leukotriene synthesis in the polymorphonuclear leukocyte and prostaglandin synthesis in platelet microsomes. The oxidation states of myeloperoxidase in the presence of H_2O_2 were also similarly affected by both P2 and NDGA. In these systems, the 4′4′-β-D-diglucopyranoside of rooperol, hypoxoside [(*E*)-1,5-bis(4′β-D-glucopyranosyloxy-3′-hydroxyphenyl) pent-4-en-1-yne; P2A] had no effect. The only system which showed significant differences in the effects of the catechols was the red blood cell. NDGA in the presence of H_2O_2 had a pronounced haemolytic effect, which did not correlate with its ability to induce methaemoglobin formation, while P2 had a much lower haemolytic effect, but stimulated the oxidation of haemoglobin to a greater extent than NDGA. NDGA is more hydrophobic than P2 which would result in a greater membrane effect. The pent-4-en-1-yne chain which links the catechol moieties in P2 can take part in the resonance structures of the semiquinone free radical, thus assisting in its stabilization and leading to increased methaemoglobin formation. This stabilization is also demonstrated by the fact that P2A affected the oxidation of haemoglobin to nearly the same extent as NDGA.

Catechols may undergo metabolic redox cycling mediated by several enzyme systems. One-electron oxidation of these molecules leads to the formation of semiquinone radicals, which can be stabilized by complexing with divalent metal ions in order to be studied by ESR spectroscopy [1, 2]. Further oxidation or disproportionation of semiquinones leads to the formation of quinones. Semiquinones and quinones can participate in a variety of reactions including covalent bonding with proteins, peptides and other molecules [3], polypeptide polymerization [4], or oxidative inhibition of enzymes such as lipoxygenases [5]. The oxidative character of catechols has also been exploited in various studies to elucidate the reaction mechanisms of peroxidases [6]. Some enzyme systems can reduce quinones and semiquinones to their corresponding catechols, e.g. xanthine oxidase and cytochrome reductases [1].

The structural formulae of the di-catechols used in this study are shown in Fig. 1. Hypoxoside [(*E*)-1,5-bis(4′β-D-glucopyranosyloxy-3′-hydroxyphenyl)pent-4-en-1-yne; P2A†] can be isolated from several members of the *Hypoxidaceae* plant family genus [7, 8]. Rooperol [(*E*)-1,5-bis(3′,4′-dihydroxyphenyl)pent-4-en-1-yne; P2] is the deglucosidated product of P2A. Nordihydroguaiaretic acid (NDGA) is a well known anti-oxidant and lipoxygenase inhibitor prepared from the evergreen desert shrub, *Larrea divaricata*. The difference in structure of the chains linking the catechol moieties in NDGA and P2 is obvious. NDGA has a 2,3-dimethyl-1,4-butanediyl bridge, while P2 has a pent-4-en-1-yne-1,5-diyl bridge. Thus P2 has a 5-carbon chain in which each π-bond system is conjugated with one of the catechol moieties and consequently possesses the potential to participate in redox reactions.

In this study NDGA, P2 and P2A were co-oxidized in the following systems of the human blood: (1) lipoxygenase in the neutrophil; (2) cyclooxygenase in platelet microsomes; (3) myeloperoxidase (MPO) in the neutrophil and (4) the erythrocyte (RBC). The effects of the catechol groups and the different linking chains in P2 and NDGA on these oxidative systems were investigated.

MATERIALS AND METHODS

Chemicals and enzymes. The sources of reagents were as follows: 1-[^{14}C]arachidonic acid, 5-[^{3}H]-HETE, 12-[^{3}H]HETE and 15-[^{3}H]HETE from Amersham International (Amersham, U.K.); leukotriene B_4 (LTB_4), prostaglandin D_2 (PGD_2), PGE_2,

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† Abbreviations: 5-HETE, 5(*S*)-hydroxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid; 15-HETE, 15(*S*)-hydroxy-5,8,11-*cis*-13-*trans*-eicosatetraenoic acid; LTB_4, leukotriene B_4; MPO, myeloperoxidase; NDGA, nordihydroguaiaretic acid; P2, rooperol or (*E*)-1,5-bis(3′,4′-dihydroxyphenyl)-pent-4-en-1-yne; P2A, hypoxoside or (*E*)-1,5-bis(4′β-D-glucopyranosyloxy-3′-hydroxyphenyl)pent-4-en-1-yne; PMN, polymorphonuclear leukocyte; PGD_2, $-E_2$, $-F_{2\alpha}$, $-G_2$ or $-H_2$, prostaglandin D_2, E_2, $F_{2\alpha}$, G_2 or H_2; RBC, erythrocyte; TXB_2, thromboxane B_2.

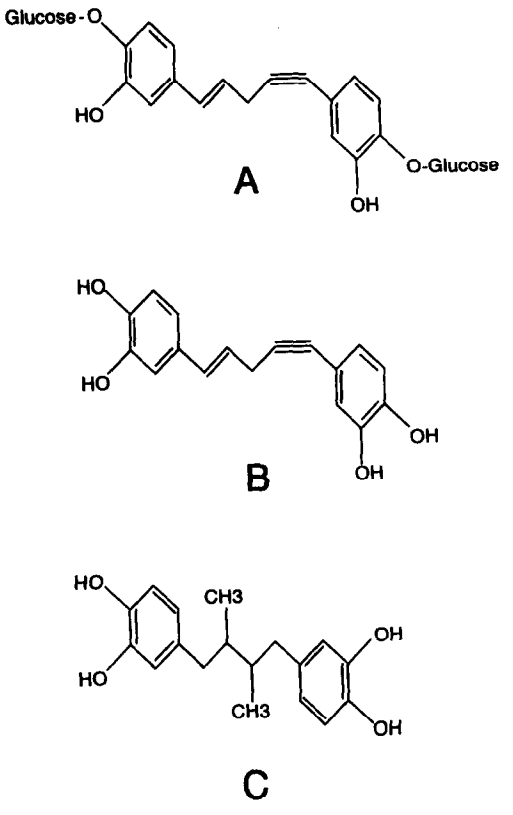


Fig. 1. Structural formulae of (A) P2A, (B) P2 and (C) NDGA.

$PGF_{2\alpha}$ and thromboxane B_2 (TXB_2), as well as NDGA, ionophore A23187, indomethacin, Histopaque-1077, RPMI 1640 tissue culture medium and glutathione from the Sigma Chemical Co. (Poole, U.K.); P2 and P2A from Essential Sterolin Products (Pty) (P.O. Box 55185, Northlands 2116, South Africa). MPO was prepared from human neutrophils as described [9] and had purity indexes (A_{423}/A_{280} nm) exceeding 0.75. All other chemicals were of the highest purity available.

Synthesis of lipoxygenase products by human polymorphonuclear leukocytes (PMNs). Human PMNs were prepared from venous blood donated by healthy volunteers receiving no medication. PMNs were isolated (>95% homogeneity) by density centrifugation on Histopaque-1077 and sedimentation through 6% dextran [10]. The final PMN pellet was suspended in RPMI-1640 medium.

Test compounds were dissolved in ethanol (P2, NDGA and indomethacin) or water (P2A) and added in 10-μL quantities to 2 mL RPMI-1640 medium containing 1×10^6 cells. After incubation for 10 min at 37°, 5 μL [^{14}C]arachidonic acid (50 μCi/mL; 56 mCi/mmol) were added. The reaction was initiated by adding 2 μL ionophore A23187 (10 mM) in dimethyl sulphoxide. After 5 min at 37°, the reaction was terminated by lowering the pH to 3 [10].

After centrifugation, the supernatant was extracted twice with 2 mL ethyl acetate. The organic phase was washed with saturated NaCl, dried over sodium sulphate and evaporated under a stream of nitrogen gas. The residue was dissolved in 60 μL methanol and subjected to TLC with diethyl ether:*n*-hexane:acetic acid (60:40:1, by vol.) as liquid phase [11].

Labelled arachidonic acid metabolites were visualized autoradiographically. ^{14}C-Radioactivity was determined in a Beckman LS5000TD liquid scintillation counter after scraping individual bands from the TLC plates. TLC plates were also exposed to iodine vapour to visualize metabolite standards.

Statistical analyses were done with the aid of the Statgraphics program (Statistical Graphics Corporation).

Synthesis of cyclooxygenase products by human platelet microsomes. Expired human platelets were obtained from the local blood bank and microsomes prepared according to Maguire and Csonka-Khalifah [12]. The final preparation was suspended in 0.05 M Tris–HCl (pH 8) containing 1 mM EDTA and had a protein concentration of 4.4 mg/mL (determined by the Lowry method [13]).

The 600 μL incubation system used for the conversion of arachidonic acid to its prostanoid metabolites contained 26 μg microsomes, 0.67 mM hydroquinone, 0.2 mM glutathione, 0.4 μM haemoglobin and the required concentration of the test compound in 0.05 M Tris–HCl. After pre-incubation for 5 min at 37°, 0.3 μCi [^{14}C]arachidonic acid (5.3 nmol) was added. The reaction was stopped after 2 min by lowering the pH to 3 with 1 N H_2SO_4. After extraction with ethyl acetate, the metabolites were separated by TLC using a chloroform:methanol:acetic acid:water mixture (90:8:1:0.5, by vol.) as liquid phase [12]. Radioactivity of individual bands was determined as described above.

Reactions with myeloperoxidase. Spectral analyses of products formed during oxidation of P2 and NDGA, and MPO-H_2O_2 adducts formed during the reaction were monitored on a Cary 219 spectrophotometer.

Oxidation of haemoglobin. RBC lysate was passed through a DEAE Sephacel column to prepare haemoglobin and its concentration determined as described [14]. Test substances (50 μM) were added to 2 mM haemoglobin in 3 mL sodium phosphate buffer (10 mM; pH 7.5). The haemoglobin spectra were recorded and the reactions initiated by the addition of H_2O_2 (final concentration 200 μM). A recording was made 2 min after the addition of H_2O_2 and subsequently at 4 min intervals. From these scans the concentration of methaemoglobin was calculated according to Szebeni *et al.* [14].

Effects on RBC. RBCs were isolated from fresh heparinized human blood as described [16]. The RBC pellet was suspended in PBS (pH 7.5). Increasing concentrations of the catechols were added to suspensions containing 1.3×10^7 cells each. After incubation at 37° for 15 min and centrifugation, the concentration of haemoglobin as well as the proportion of methaemoglobin in the supernatant was determined [14]. Methaemoglobin was also determined in the supernatants of hypotonically

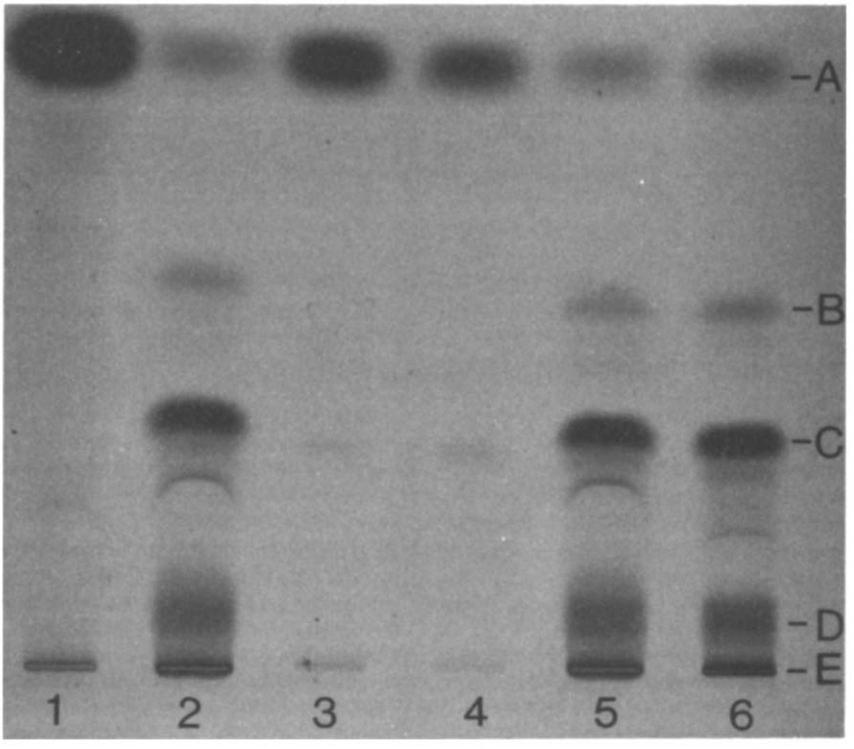


Fig. 2. Autoradiogram of TLC of extracts of PMNs incubated with [^{14}C]arachidonic acid. Experimental details are described in Materials and Methods. Lane 1 represents [^{14}C]arachidonic acid incubated with boiled PMNs. All other lanes represent incubations of viable PMNs with the following test substances: lane 2, 10 μL ethanol; lane 3, 10 μM NDGA; lane 4, 10 μM P2; lane 5, 10 μM P2A; lane 6, 20 μM indomethacin. The positions of unlabelled standards, detected after exposure to iodine vapour, are indicated on the right: (A) arachidonic acid; (B) 15-HETE; (C) 5-HETE; (D) LTB_4; (E) origin.

lysed intact cells recovered from the catechol reaction mixture.

RESULTS

Inhibition of PMN lipoxygenase activity

Figure 2 shows a TLC autoradiogram of arachidonic acid metabolites extracted from the incubation medium of PMNs stimulated with ionophore A23187 in the presence of [^{14}C]arachidonic acid. Metabolites which could be identified are indicated. No metabolites were detected when arachidonic acid was incubated with boiled PMNs (lane 1). Indomethacin (20 μM) did not suppress the synthesis of metabolites (lane 6). It is also clear that 10 μM P2A had little effect (lane 5). NDGA or P2 (both at 10 μM) completely suppressed the synthesis of all the metabolites (lanes 3 and 4).

The effects of increasing concentrations of P2, NDGA and P2A on the synthesis of 15-HETE and LTB_4 derived from [^{14}C]arachidonic acid are shown in Fig. 3. Figure 3A demonstrates a stronger inhibition of 15-HETE synthesis by P2 (IC_{50} = 0.7 μM) than by NDGA (IC_{50} = 1.8 μM). The differences between these sets of data were significant at the 95% confidence level. The differences in the effects of P2 and NDGA on LTB_4 synthesis were not significant (IC_{50} = 0.4 μM; Fig. 3B). The inhibition curves for 5-HETE were similar to those of Fig. 3B for LTB_4. P2A had little effect up to a concentration of 200 μM.

Inhibition of platelet microsome cyclooxygenase activity

Figure 4 demonstrates the effects of NDGA, P2 and indomethacin on arachidonic acid metabolism by platelet microsomes. As expected, synthesis of the prostanoids was highly sensitive to indomethacin and IC_{50} values of 0.1 μM for TXB_2 and PGD_2, and 0.8 μM for $PGF_{2\alpha}$ could be calculated. The inhibition curves of each prostanoid were similar for P2 and NDGA. TXB_2 synthesis was most strongly inhibited (IC_{50} = 17 μM; A), followed by PGD_2 (IC_{50} = 52 μM; B) and $PGF_{2\alpha}$ (IC_{50} = 70; C). At low catechol concentrations $PGF_{2\alpha}$ synthesis was stimulated (about three-fold at 50 μM), followed by a sharp decline in synthesis at higher catechol concentrations. Similar trends in the syntheses of TXB_2 and PGD_2 were observed (A and B), although the stimulatory phases of the curves were less obvious than in the case of $PGF_{2\alpha}$. The inhibition curves of PGE_2 were similar to those of PGD_2.

Reactions with MPO

The addition of H_2O_2 to a mixture of MPO and P2 resulted in an absorbance peak at 355 nm for the

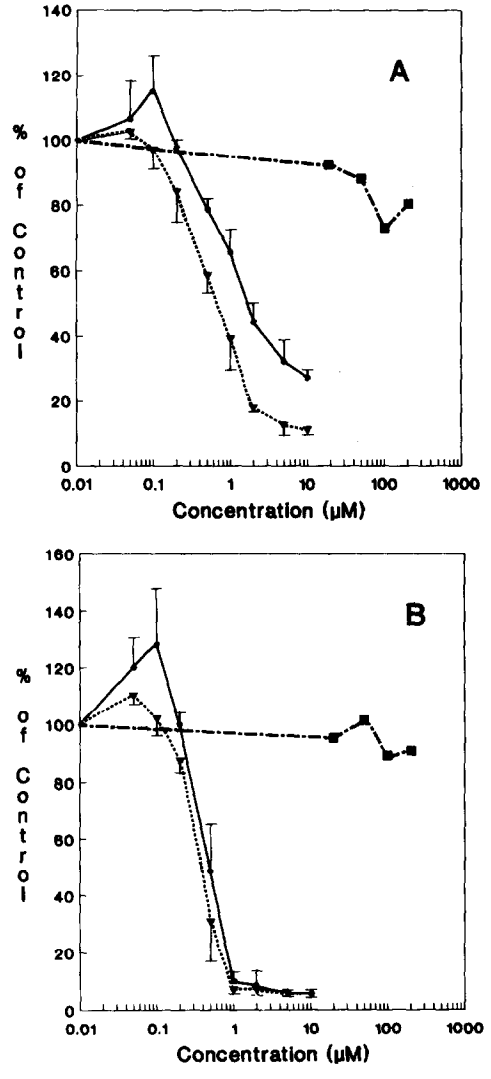


Fig. 3. Effects of P2 (▼···▼), NDGA (●——●) and P2A (■—·—·—■) on the synthesis of lipoxygenase products derived from [^{14}C]arachidonic acid incubated with PMNs as described in Materials and Methods. (A) Effects on 15-HETE synthesis; (B) effects on LTB_4 synthesis. Data are expressed as percentages of the control and represent the means ± SD of three different experiments.

oxidation product of the di-catechol (Fig. 5A). The peak increased in intensity with time (not shown) and also with serial additions of H_2O_2 up to 323 μM, when it started to decrease again. The decrease in absorbance coincided with the appearance of turbidity in the solution. NDGA reacted similarly, although the equivalent peak at 355 nm was lower. Also, no drop in absorbance during serial H_2O_2 additions and no turbidity were observed (Fig. 5B).

Addition of H_2O_2 to MPO caused the typical shift in the Soret band, as well as the appearance of an absorbance peak at 630 nm (Fig. 6A and B; scans 2). When P2 (or NDGA) was added 30 sec after addition of H_2O_2 and the recording was made 2.5 min later, no evidence of the peak at 630 nm was visible (Fig. 6B; scan 3). Addition of P2 prior to H_2O_2 resulted in no shift in the Soret peak and no

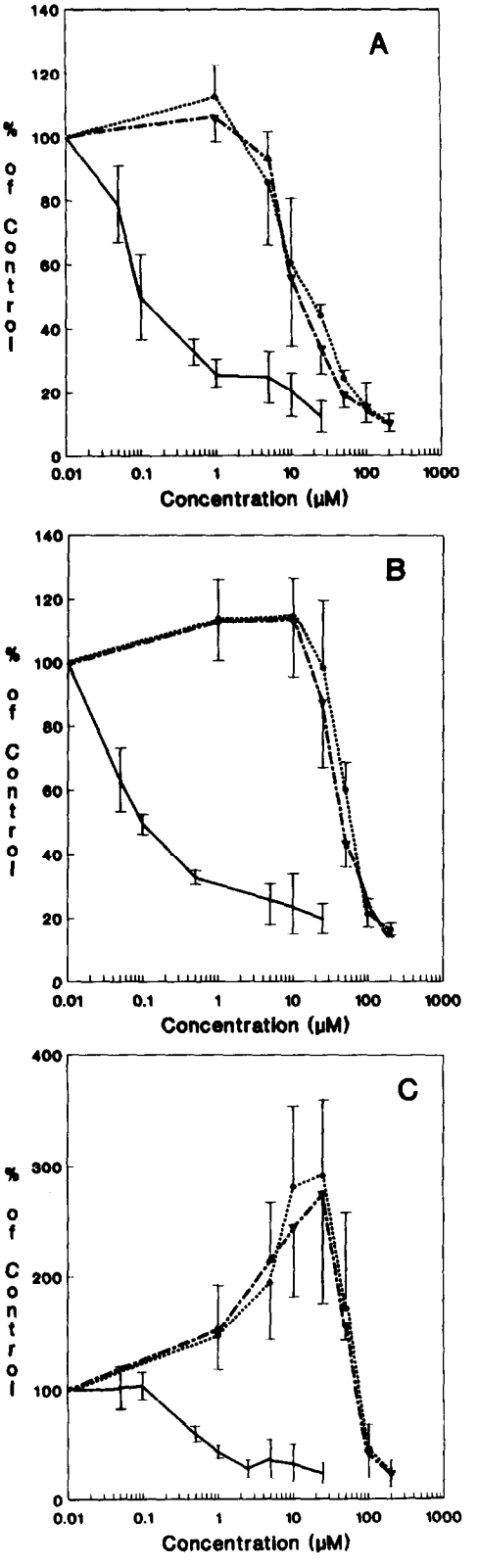


Fig. 4. Effects of P2 (▼—·—▼), NDGA (●—·—●) and indomethacin (————) on the cyclooxygenase products derived from [^{14}C]arachidonic acid incubated with platelet microsomes as described in Materials and Methods. The metabolites quantitated were TXB_2 (A), PGD_2 (B) and $PGF_{2\alpha}$ (C). Data are expressed as percentages of the control and represent means ± SD of three different experiments.

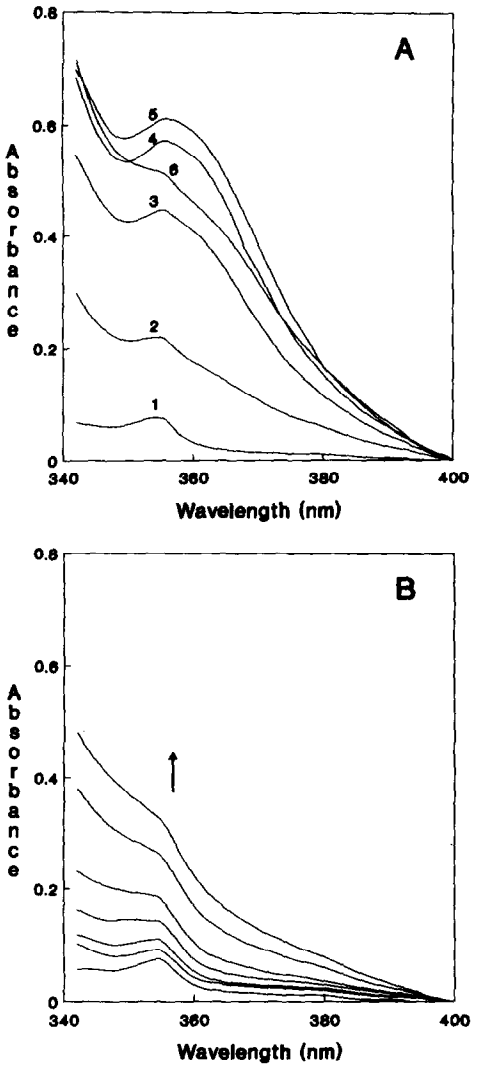


Fig. 5. Absorbance spectra of 50 μM P2 (A) and 50 μM NDGA (B) recorded during reactions of 2.3 μM MPO with H_2O_2 in 10 mM phosphate buffer pH 7.5 (total volume 3 mL). Scan 1 in A and the corresponding scan in B are spectra of catechol solutions in the presence of enzyme only. Scans of the solutions after serial additions of 20 μL 10 mM H_2O_2 are indicated by 2–6 in A and corresponding spectra in B. Spectra were recorded immediately after the addition of H_2O_2. Reference cuvettes contained the same concentration of MPO without the catechols. Experiments were repeated at least three times with no qualitative differences in the results.

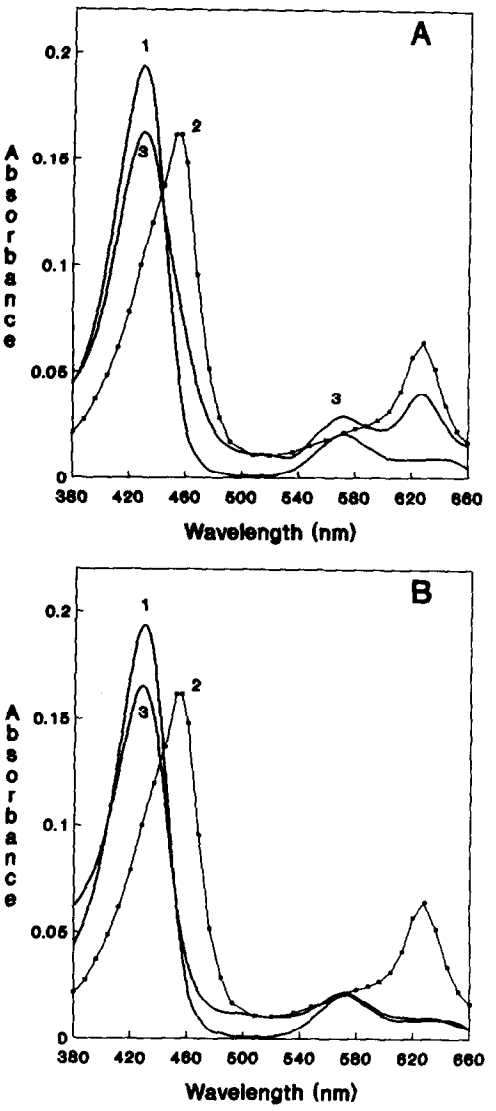


Fig. 6. Spectral analysis of MPO. (A) Scans 1, 2.3 μM MPO; 2, 30 sec after adding H_2O_2 (100 μM in mixture); 3, 2.5 min later. (B) Scans 1 and 2, same as in A; 3, P2 (50 μM in mixture) was added 30 sec after adding the H_2O_2 and the recording was made 2.5 min later. The reactions were done in 3 mL 10 mM phosphate buffer, pH 7.5. The reference cuvettes contained only buffer. Experiments were repeated at least three times with no qualitative differences in the results.

formation of the peak at 630 nm. Without catechol supplementation, a considerable amount of MPO–H_2O_2 adducts were still present after 3 min (Fig. 6A; scan 3). P2 and NDGA reacted similarly in this system, while P2A had only a minor effect on the decay of the MPO–H_2O_2 adducts.

Oxidation of haemoglobin

The relative abilities of P2, P2A and NDGA (the final concentration in each case was 50 μM) to oxidize 2 mM haemoglobin in the presence of 200 μM H_2O_2 are shown as increasing methaemoglobin, calculated from the absorbance curves of the haemoglobin in each incubation mixture (Fig. 7). It is evident that P2 had the highest ability to promote oxidation of haemoglobin by H_2O_2, followed by NDGA and P2A, whose abilities were noticeably higher than that of the control.

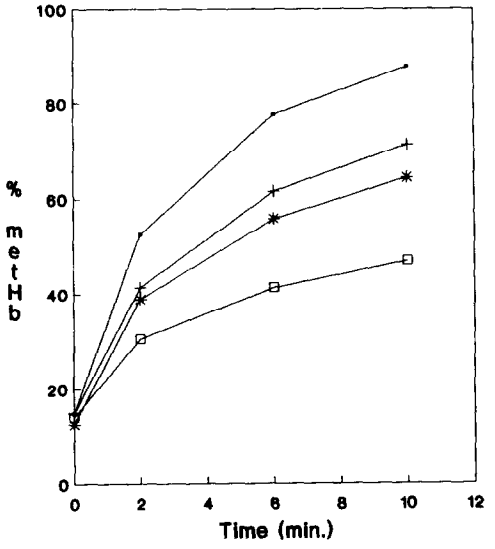
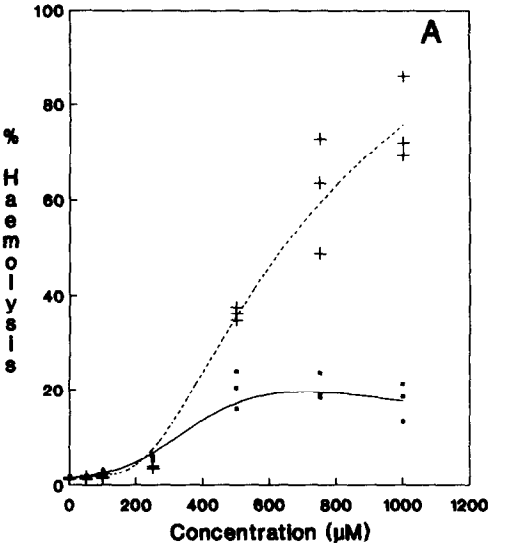


Fig. 7. Effect of the catechols on methaemoglobin formation. Data points were calculated from the absorbtion curves recorded as described in Materials and Methods. P2A (*); P2 (■); NDGA (+); control (□).



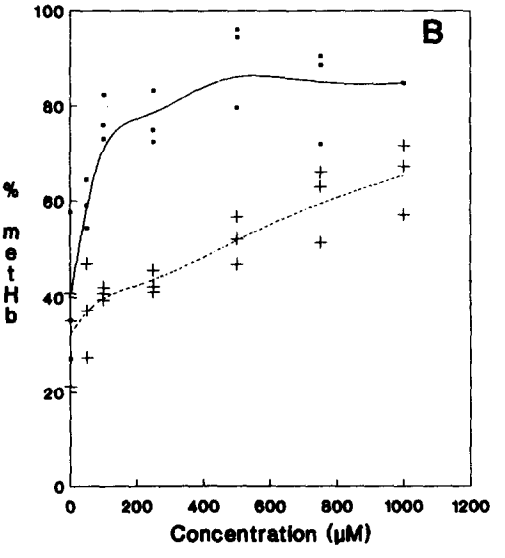


Fig. 8. Relationship between catechol-induced haemolysis and haemoglobin oxidation in RBCs in PBS pH 7.5. P2 (■———■) and NDGA (+———+). (A) Haemolysis as a function of catechol concentration. Reaction mixtures were centrifuged and haemoglobin determined in the supernatants as described in Materials and Methods. (B) Methaemoglobin formation as a function of catechol concentration. The proportion of methaemoglobin in the supernatant was determined spectrophotometrically [14]. Methaemoglobin proportions in the surviving cells showed similar trends.

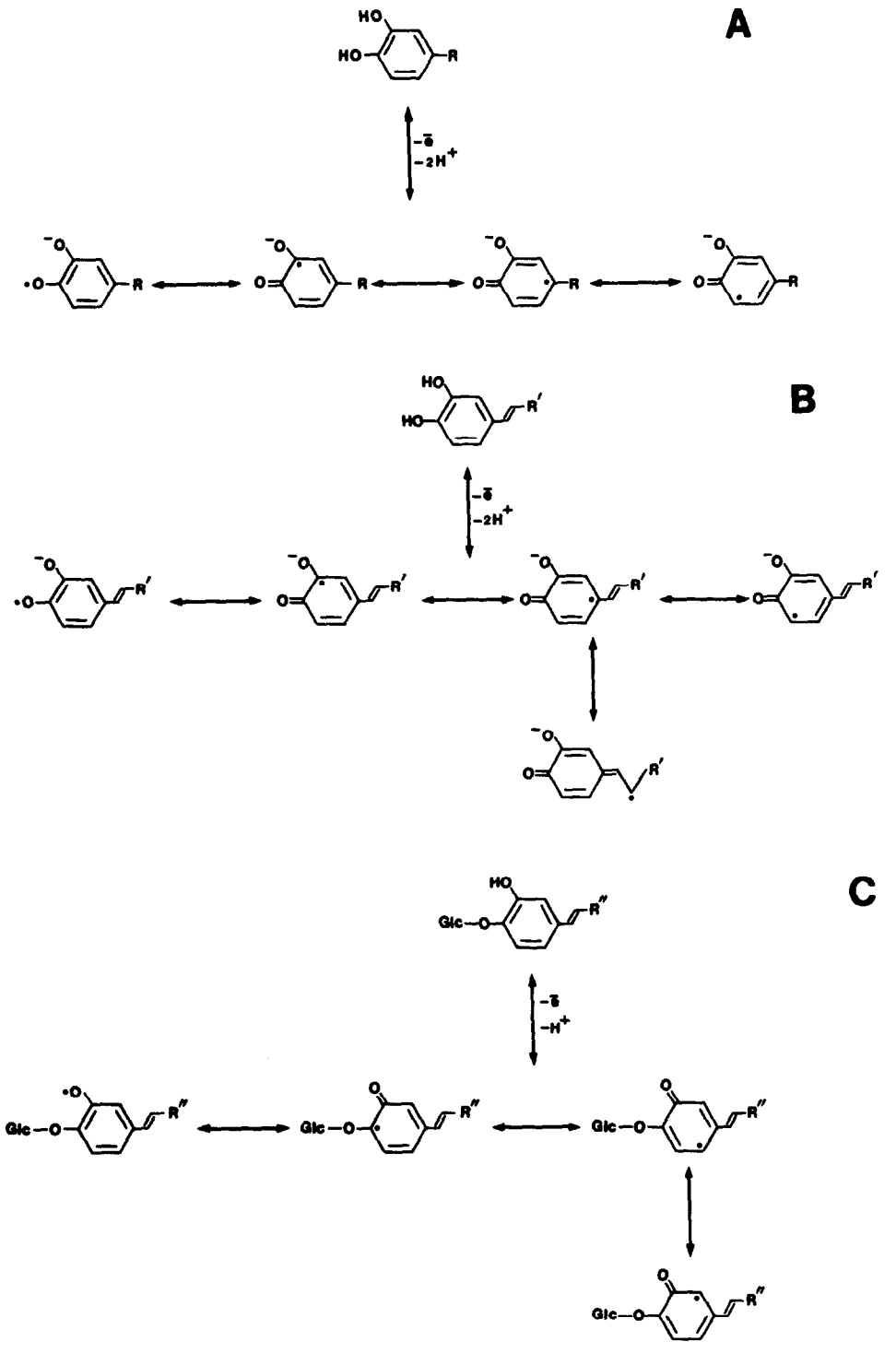
Effect on RBC

The haemolytic effects of P2 and NDGA are shown in Fig. 8. P2A had no effect on the integrity of the cells at concentrations of up to 1 mM (not shown). Above concentrations of 100 μM catechol, an increase in haemolysis occurred. Total haemolysis occurred in the presence of 1 mM NDGA. In the presence of P2, haemolysis reached a maximum of about 20% after which it tended to decrease again (A). Figure 8B shows that the drastic haemolytic effect of NDGA did not correlate with its ability to induce methaemoglobin formation. Methaemoglobin determined in the supernatant following catechol-induced haemolysis is shown in Fig. 8B, but methaemoglobin proportions in the surviving cells showed similar trends. Recovery of total haemoglobin was achieved by hypotonic lysis of the RBCs and no significant differences were found between the amounts of haemoglobin in the cell incubations containing NDGA and P2.

DISCUSSION

To evaluate the effects of P2 and NDGA on the different oxidative systems used in this study, the shape, hydrophobic character and redox potential of the different molecules should be taken into account. Similar effects of P2 and NDGA in some of the test systems are expected, although their redox potentials should differ significantly from each other. Although the methyl substituent groups of the NDGA chain will impose a certain degree of rotational hindrance, the two catechol groups are identical and benefit from an electron donating effect of the bridge alkanyl substituent. The C_5-bridge system in P2 on the other hand is very rigid due to conjugational alignments.

In the lipoxygenase and cyclooxygenase systems, NDGA and P2 showed similar effects (Figs 2–4). In the case of 15-HETE inhibition, P2 had more than twice the potency of NDGA (0.7 against 1.8 μM). It has been reported that NDGA inhibits soybean lipoxygenase by reducing the catalytically active ferric lipoxygenase to its inactive ferrous form [5, 15]. This reduction results in the formation of semiquinone radicals which can react further to form *o*-quinones.



Scheme 1. Possible resonance structures of the semiquinone radical of NDGA (A), P2 (B) and P2A (C).

Both P2 and NDGA formed coloured products with absorbance peaks at 355 nm during cooxidation in the MPO system (Fig. 5). These peaks gradually flattened out with time and the spectra became continuous, indicative of polymerization [16], for which quinone formation is a prerequisite.

The electron-donatory properties of the di-catechols are also demonstrated by their effects on

the synthesis of prostanoids by the cyclooxygenase system of the human platelet (Fig. 4). Synthesis of TXB_2 from PGH_2 requires cytochrome P450 [17], which could have been inhibited by the catechol. This would result in a stronger inhibition of thromboxane synthesis relative to the syntheses of the other prostanoids. With $PGF_{2\alpha}$ and, to a lesser extent, PGE_2 and PGD_2 syntheses, the peaks of stimulation coincided with concentrations where TXB_2 was inhibited. This phenomenon was also reported by Salmon [11] for the inhibition of TXB_2 synthesis in platelets by benzyl imidazole. However, higher concentrations of the catechols, unlike benzyl imidazole, also inhibited PGE_2, PGD_2 and $PGF_{2\alpha}$ syntheses, indicating no specific inhibition of TXB_2 synthesis. This suggests involvement of the catechols earlier in the cyclooxygenase chain.

During prostaglandin synthesis, PGG_2 is converted into PGH_2 by the peroxidative activity of cyclooxygenase. Similar to other peroxidases, hydroperoxides form complexes with this peroxidase to alter the redox states of its haem iron. Some of the spectral changes resulting from alteration in the redox states of a peroxidase are illustrated for MPO in Fig. 6. On addition of H_2O_2 to the enzyme, native ferric MPO is converted to the highly active Compound I. In the absence of a suitable substrate, the unstable Compound I undergoes a one-electron reduction step to form the less reactive oxoferryl ($O = Fe^{IV}$) MPO or Compound II. Compound III, the perferryl form of the peroxidase, is similar to oxygenated haemoglobin and can be formed by reaction of Compound II with H_2O_2 [18]. Although Compound III can react with a number of electron donors, including catechols, it is normally catalytically inactive. The spectra illustrated in Fig. 6 (scans 2 of A and B) are most likely a mixture of the unstable oxoferryl($O = Fe^{IV}$)MPO and its perferryl (O_2Fe^{IV})MPO reaction product with H_2O_2 [18]. Thus, in the presence of electron donors such as the catechols, accumulation of the less reactive Compounds II and III will be prevented and Compound I reduced by two electrons to the resting ferric state. The same mechanism should apply to the cyclooxygenase system. At low concentrations the catechols act as redox catalysts in the conversion of PGG_2 to PGH_2, while at higher concentrations they may act as competitive substrates during cooxidation with PGG_2 which will result in inhibition of prostanoid synthesis.

In contrast to the effects of NDGA and P2 on lipoxygenases and cyclooxygenases, P2A had a much lower potency. Similar results were reported for the coumarin esculetin and its glucoside esculin [11], emphasizing the importance of unblocked catechol hydroxyls. The anti-lipoxygenase activities of catechol flavanoids were also decreased by methylation of their catechol groups [19].

Although both P2 and NDGA possess two catechol moieties each, there was a significant difference in their abilities to stimulate haemoglobin oxidation in the presence of H_2O_2 in a cell-free system and in the intact RBCs without H_2O_2 supplementation (Figs 7 and 8B, respectively). The first step in the mechanism of methaemoglobin formation is H_2O_2-induced oxidation of haem iron to its ferric form. Further complexation with H_2O_2 leads to the formation of ferryl haemoglobin (Fe IV) which is equivalent to Compound II of peroxidases. Ferryl haemoglobin can oxidize the catechol in a one-electron oxidation step to the corresponding semiquinone with reduction of the ferryl to the Fe III of methaemoglobin. *o*-Semiquinones are transient, decaying rapidly via disproportionation to give the catechol and *o*-quinone [20].

Resonance structures of the semiquinone radical in the phenolic rings of NDGA and P2 are illustrated in Scheme 1. The NDGA-semiquinone is resonance stabilized in the ring structure (A), while the radical in the P2 ring can be further stabilized by participation of the conjugated double bond in the side chain, to form an alkyl quinone radical (B). Thus, a direct oxidation of the ferrous iron of oxygenated haemoglobin by a *o*-semiquinone radical is more likely with P2 than with NDGA. This may explain, at least in part, the higher capability of P2 to generate methaemoglobin. The relative ability of NDGA to oxidize haemoglobin in the presence of H_2O_2 was comparable to that of P2A which has only two of its four hydroxyl groups blocked and is therefore still a good electron donor (Scheme 1C). In addition, the radical generated in the P2A ring following one-electron oxidation may also be resonance stabilized in the conjugated side chain (Scheme 1C).

In contrast to the experiments with isolated haemoglobin, the RBCs were not supplemented with H_2O_2, but in both cases the mechanism of methaemoglobin formation should be similar. The initiating step of the catechol autoxidation reaction appears to involve electron transfer [1], a reaction which is stimulated at alkaline pH:

$$\text{Catechol} + O_2 \xrightarrow{-2H^+} o\text{-Semiquinone} + O_2^{-\cdot}.$$

Reactions of superoxide with the catechol will yield H_2O_2. It is noticeable that the ability of P2 to generate methaemoglobin in the lysate of RBCs did not correlate with its ability to induce haemolysis.

According to Fig. 8A, NDGA is a considerably stronger haemolytic agent than P2. An explanation for this rather conflicting result could be that NDGA can attain higher levels in the membrane than P2, since it should be more lipid soluble.

Although the activity of P2 in biological systems can largely be explained by the catechol moieties of the molecule, the nature of the bonds in the carbon chain linking the catechol groups can influence the activity of the compound. Stabilization of the semiquinone by resonance with the double bonds of the linking chain affords a molecule with higher oxidative capacity in some systems in the blood.

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