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SEMI-QUINONE FORMATION FROM THE CATECHOL AND ORTHO-QUINONE METABOLITES OF THE ANTITUMOR AGENT VP-16-213

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The catechol metabolite of the antitumor agent VP-16-213 and the ortho-quinone of VP-16-213 – a secondary metabolite formed from the catechol – easily undergo auto-oxidation into a free radical at pH $\geqslant$ 7.4. By elevation of the pH from 7.4 to 10, an increase in the production of the free radical was observed, which was accompanied by the formation of products with higher hydrophylicity than the catechol and ortho-quinone, as found by HPLC-analysis. The hyperfine structure of the free radical indicates that it is the semi-quinone radical of VP-16-213. At pH 12.5 a secondary radical is formed from the catechol and the ortho-quinone of VP-16-213 besides the semi-quinone radical. One-electron oxidation of the catechol with horseradish peroxidase/hydrogen peroxide resulted in the formation of the same radical as observed under alkaline conditions and subsequent oxidation to the ortho-quinone. If the ortho-quinone was incubated with NADPH cytochrome P-450 reductase, a free radical was detected by spin-trapping with POBN, but not without spin-trapping.

Studies on inactivation of ΦX174 DNA by the system ortho-quinone of VP-16-213/NADPH cytochrome P-450 reductase suggest that the semi-quinone radical may play a role in the process of inactivation of DNA.

KEY WORDS: VP-16-213, catechol, ortho-quinone, semi-quinone, free radical.

ABBREVIATIONS USED: ESR, electron spin resonance spectroscopy; HPLC, high performance liquid chromatography; ss, single-stranded; 4-POBN, α-4-pyridyl-1-oxide N-tert-butyl nitrone, DTPA, diethylene triamine pentaacetic acid; SOD, superoxide dismutase; HRP, horseradish peroxidase.

INTRODUCTION

The precise mechanism of action of the important antineoplastic agent VP-16-213 [4′-demethylepipodophyllotoxin-9- (4,6-O-ethylidene-β-D-glucopyranoside), NSC 141540, Fig. 1] is unknown. The cytotoxicity of VP-16-213 is probably caused by DNA damage.[1,2] Two possible explanations for the mechanism of DNA inactivation caused by VP-16-213 are presented in literature. Many indications were obtained that

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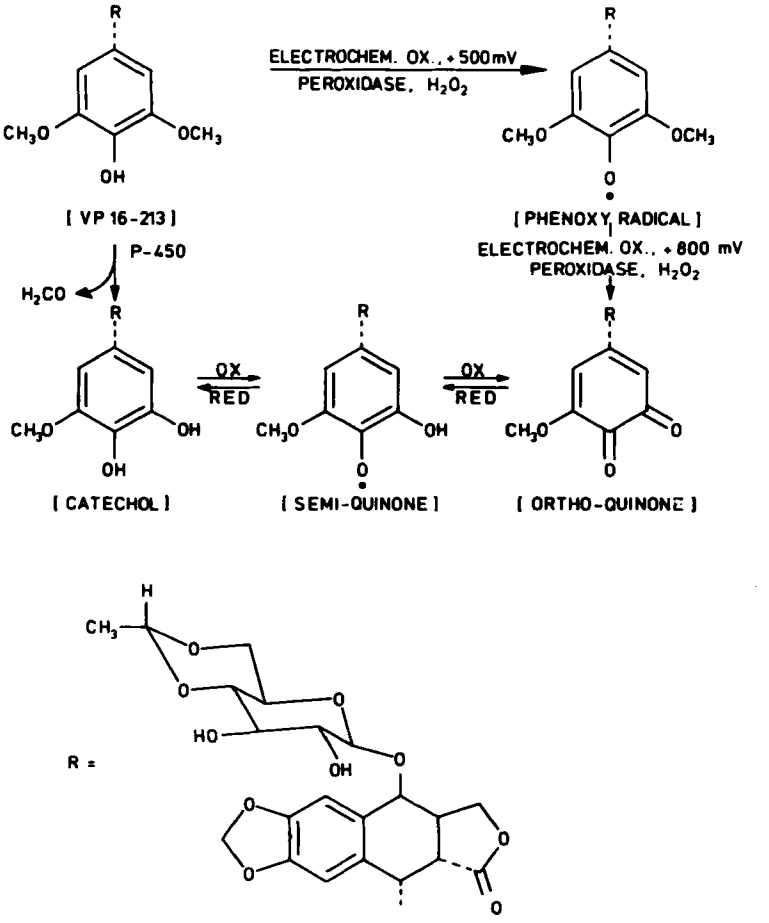

FIGURE 1 Proposed conversions of the dimethoxyphenol ring of VP-16-213.

the induction of at least a part of the DNA-lesions can be explained by the ability of VP-16-213 to interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II by stabilizing a cleavable complex.[3] Therefore, it has been proposed that the mechanism of the cytotoxic action of VP-16-213 is mainly the "poisoning" of DNA topoisomerase, probably by its ability to bind reversibly to this enzyme. Another hypothesis is that the dimethoxyphenol ring of VP-16-213 (the E-ring) is metabolized to products which cause DNA damage. This hypothesis was presented by Loike and Horwitz, who observed that the presence of the 4'-OH group in the E-ring is necessary for DNA inactivation and that isolated purified DNA is not broken down by the parent drug.[4] We reported that VP-16-213 undergoes O-demethylation by cytochrome P-450 to the ortho-dihydroxy derivative or catechol of VP-16-213[5,6] and that VP-16-213 can be converted into a phenoxy radical by oxidation of the dimethoxyphenol ring.[7] O-demethylation of VP-16-213 to the catechol was recently also reported by Haim *et al.*[8] Oxidation of the catechol of VP-16-213 leads to the formation of the ortho-quinone of VP-16-213,[6] a product which is also

formed on peroxidation of the drug.[9] The catechol and ortho-quinone were found to inactivate ΦX174 DNA, in contrast to VP-16-213 itself and its phenoxyradical.[10] These observations supported the hypothesis that DNA-inactivation by VP-16-213 is caused by activation of the dimethoxyphenol ring.

The possible conversions of the dimethoxyphenol ring of VP-16-213 are shown in Fig. 1. A possible intermediate between the catechol and ortho-quinone metabolites of VP-16-213 is the semi-quinone free radical. The aim of the present study was to investigate the possible formation of the semi-quinone radical from both the catechol and the ortho-quinone of VP-16-213, and the possible role of the formation of the semi-quinone radical in the inactivation of DNA by the catechol and the ortho-quinone of VP-16-213.

MATERIALS AND METHODS

Drugs and chemicals

VP-16-213 was a gift from the Bristol-Myers Company (Syracuse, N.Y., U.S.A.). The ortho-quinone of VP-16-213 was synthesized by controlled potential electrolysis of VP-16-213 at a Pt-gauze electrode.[11] The ortho-dihydroxy derivative of VP-16-213 (the catechol) was synthesized from the ortho-quinone of VP-16-213 by reduction with ascorbic acid.[7] 4-POBN and DTPA were purchased from Janssen Chimica, Beerse, Belgium. Superoxide dismutase, catalase and NADPH were purchased from Boehringer Mannheim GmbH, GFR. Horseradish peroxidase type II (specific activity 190 units/mg) was purchased from Sigma Chemical Company, St. Louis, MO., U.S.A. Cytochrome P-450 reductase was purified from phenobarbital-induced rat liver microsomes by the method described by Waxman *et al.*[12] The purified cytochrome P-450 reductase has a specific activity of 24 units/mg protein. All other chemicals used were reagent grade.

Incubations with ss ΦX174 DNA

Single-stranded ΦX174 DNA was isolated from wild-type ΦX174 DNA bacteriophage according to Blok *et al.*[13] Solutions of ss ΦX174 DNA (1.7×10^{-1} μg/ml) in 5×10^{-2} M potassium phosphate pH 7.4 were incubated at 37°C for increasing periods of time with (a) VP-16-213 (1.8×10^{-4} M), (b) the ortho-quinone of VP-16-213 (1.8×10^{-4} M), NADPH (1×10^{-3} M) and cytochrome P-450 reductase (0.31 units) in absence or presence of 0.5% DMSO. At several time intervals of incubation, samples of 25 μl were taken and diluted twentyfold with icecold 0.025 M Tris-HCl buffer (pH 8.2) to stop the reaction. The biological activity of ΦX174 DNA was determined by measuring the bacteriophage production after transfection of the DNA to E. coli spheroplasts in essentially the same way as described by Lutgerink *et al.*[14] The *E. coli* strain used for the transfection assay was AB1157 (wild type).[15]

High performance liquid chromatography

HPLC was performed using a Perkin Elmer LC2 HPLC pump with a Perkin Elmer LC-75 Spectrophotometric Detector at $\mu = 254$ nm, a Waters U6K injector and a Waters μBondapak phenyl column (10 μm, 300 × 4.6 mm), at a flow rate of 1.2 ml/min. The mobile phase consisted of methanol/ 0.025 M phosphate buffer (pH 4.5,

50/50 v/v%). The catechol of VP-16-213 (3.5×10^{-4} M) was dissolved in aqueous solutions of pH 8.5, 9, 10, 11 or 12.5 and the solutions were immediately subjected to HPLC. Incubations of the catechol or ortho-quinone of VP-16-213 (3.5×10^{-4} M) were performed in 2×10^{-2} M potassium phosphate pH 7.4 or pH 8.5 at 37°C for 1 hour and 30 minutes, respectively. After incubation, the samples were kept at room temperature for 24 hours and subjected to HPLC.

Incubation of the catechol of VP-16-213 (1.7×10^{-4} M) with 5×10^{-2} M phosphate buffer pH 7.4, 1×10^{-3} M H_2O_2 and 3.8 unit HRP was performed at 37°C in a total volume of 1 ml. After 0, 10, 20 and 30 min of incubation, 200 μl samples were taken for HPLC analysis. The ortho-quinone of VP-16-213 (1.3 or 2.6×10^{-4} M) was incubated at 37°C with 5×10^{-2} M potassium phosphate pH 7.4, 1×10^{-3} M NADPH, cytochrome P-450 reductase (0.5 unit/ml) and the spin trap POBN (1×10^{-2} M) for $3\frac{1}{2}$ hours in a total volume of 0.5 ml. At several time intervals of incubation, samples were taken for HPLC and ESR analysis. For the analysis of these enzymatic incubations, the HPLC system consisted of a Waters M45 Solvent Delivery System in combination with a Perkin-Elmer LC-75 Spectrophotometric Detector at $\mu = 254$ nm and a CP-Microspher 3 μm C18 column (100×4.6 mm). The flow rate was 0.7 ml/min and the mobile phase consisted of methanol/water (40/60 v/v%). This system could separate the ortho-quinone and catechol of VP-16-213.

Electron spin resonance spectroscopy

ESR spectra were recorded at room temperature on a Varian E-3 spectrometer with an E-4531 multipurpose cavity. The modulation frequency of the spectrometer was 100 kHz. Instrumental conditions are described in legends to figures.

The catechol and ortho-quinone of VP-16-213 (3.5×10^{-4} M) were dissolved in aqueous solutions of pH 8.5, 9, 10, 11 or 12.5 and subjected immediately to ESR. Incubations of the catechol and ortho-quinone of VP-16-213 (3.5×10^{-4} M) were performed in 2×10^{-2} M potassium phosphate pH 7.4 or pH 8.5 at 37°C for 1 hour. At 0 and 30 minutes, samples were taken for ESR analysis. The incubation mixture of the catechol with horseradish peroxidase/ hydrogen peroxide (see high performance liquid chromatography) was subjected to ESR analysis after 0, 5 and 30 min of incubation.

The ortho-quinone of VP-16-213 (1.3 or 2.6×10^{-4} M) was incubated at 37°C with 5×10^{-2} M potassium phosphate pH 7.4, 1×10^{-3} M NADPH, cytochrome P-450 reductase (0.5 unit/ml) and the spin trap POBN (1×10^{-2} M) for $3\frac{1}{2}$ hours in a total volume of 0.5 ml. At several time intervals of incubation, samples were taken for ESR analysis. Effects of incubation conditions were studied by adding DMSO (2×10^{-1} M), SOD (2.5 μg/ml), catalase (1000 units/ incubation) or DTPA (5×10^{-3} M), to the incubation medium.

RESULTS

A. Effect of the pH on the stability of the catechol and ortho-quinone of VP-16-213 in aqueous solutions

If the catechol of VP-16-213 was dissolved in an aqueous solution of pH 8.5, an ESR signal was immediately detected (Fig 2A), which was still present after 30 minutes. The hyperfine structure of the signal showed 12 lines, the linewidth was 6.6 Gauss and

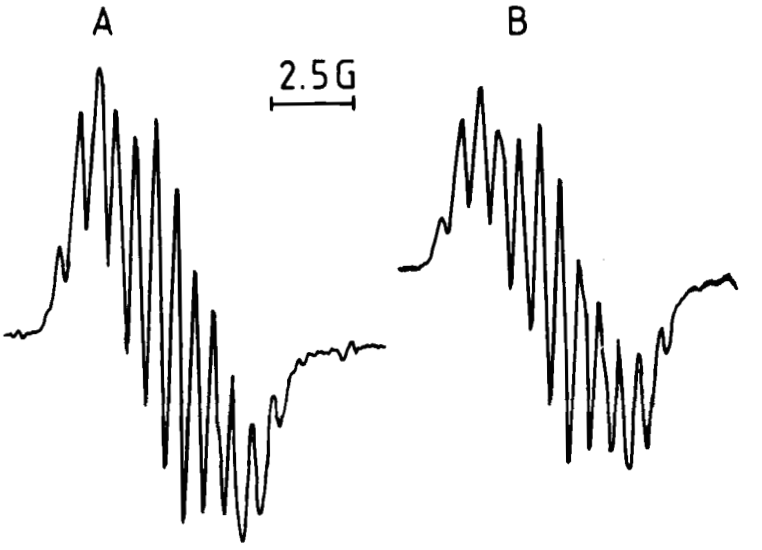

FIGURE 2 Free radical obtained after dissolution of the catechol (A) or the ortho-quinone (B) of VP-16-213 in aqueous solution of pH 8.5. Instrumental conditions were as follows: magnetic field, 3380 G; scan range, 50 G; modulation amplitude, 0.4 G; gain, 1.5×10^5; power, 32 mW; scan time, 16 min; time constant, 1 sec.

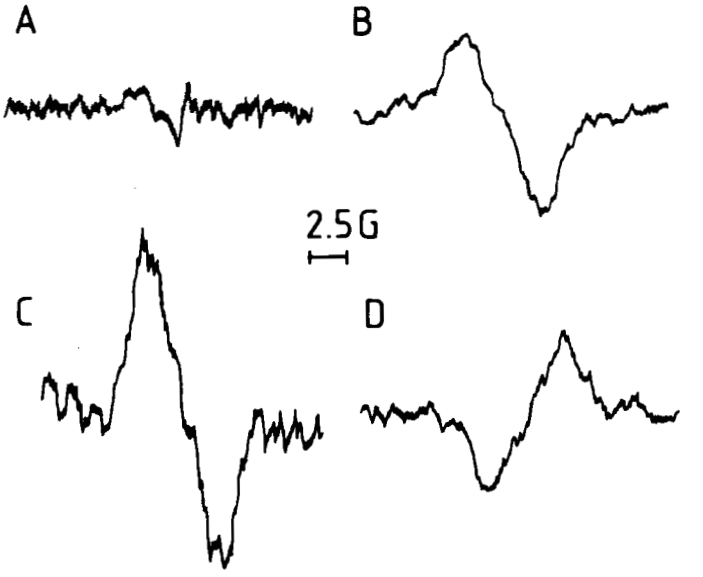

FIGURE 3 Free radical obtained after incubation of the catechol (A, t = 0 and B, t = 30 min) and the ortho-quinone (C, t = 0 and D, t = 30 min) at 37°C in phosphate buffer pH 7.4. Instrumental conditions were as follows: magnetic field, 3379 G; scan range, 50 G; modulation amplitude, 2 G; gain, 1.25×10^6; power, 32 mW; scan time, 8 min; time constant, 3 sec.

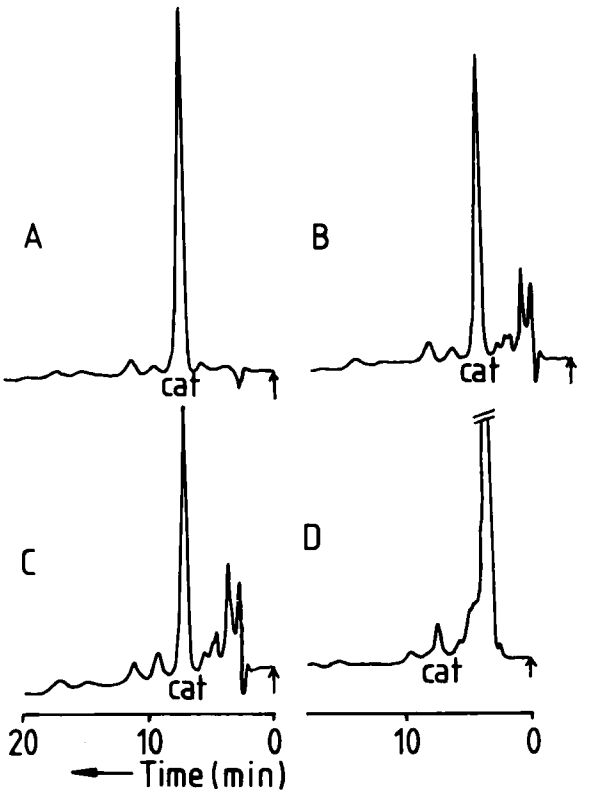

FIGURE 4 HPLC-analysis of the decomposition of the catechol of VP-16-213 (cat) in aqueous solutions of pH 9 (A), 10 (B), 11 (C) and 12.5 (D).

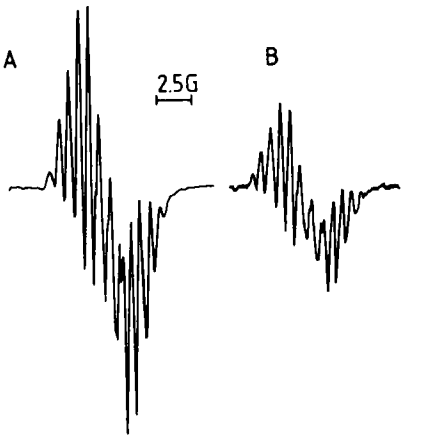

FIGURE 5 Free radical obtained after dissolution of the ortho-quinone (A) or the catechol (B) of VP-16-213 in aqueous solution of pH 12.5. Instrumental conditions were as described in the legend to Figure 3, except the gain was 8×10^4.

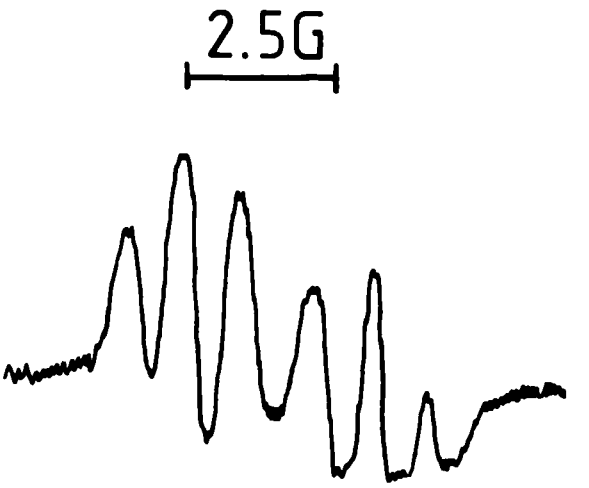

FIGURE 6 Free radical obtained from the ortho-quinone of VP-16-213 after 30 min at pH 12.5 and 20°C. Instrumental conditions were as follows: magnetic field, 3380 G; scan range, 15 G; modulation amplitude, 1 G; gain, 1×10^6; power, 100 mW; scan time, 40 sec; time constant, 10 msec.

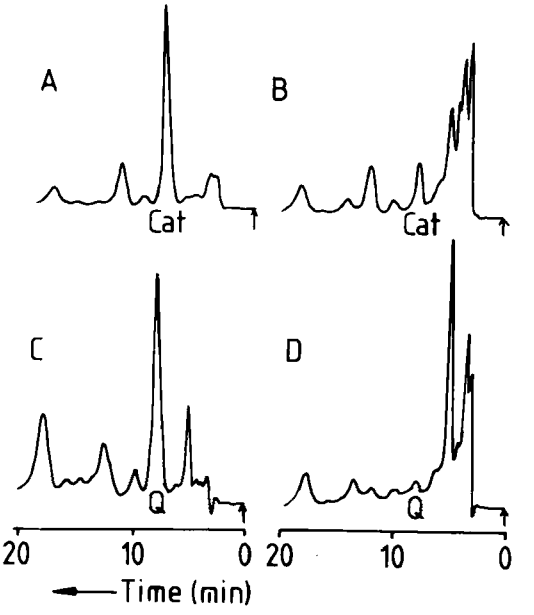

FIGURE 7 HPLC-analysis of the decomposition of: A. Catechol (Cat) at pH 7.4 for 1 hour at 37°C and 24 hours at 20°C. B. Catechol at pH 8.5 for 1 hour at 37°C and 24 hours at 20°C. C. Ortho-quinone (Q) at pH 7.4 for 30 min at 37°C and 24 hours at 20°C. D. Ortho-quinone at pH 8.5 for 30 min at 37°C and 24 hours at 20°C.

the g-value 2.003. In contrast to incubation at pH 8.5, incubation of the catechol at 37°C in phosphate buffer of pH 7.4 resulted in a weak ESR signal (Fig 3A, B). On elevation of the pH of the solutions (pH 8.5–12.5), an increase in the formation of the free radical was observed. In reversed phase HPLC analysis, it was found that this was accompanied by the formation of products with shorter retention time, thus higher hydrophylicity (Fig 4). The main product formed at pH 12.5 had a very short retention time. The ESR signal recorded for the catechol solution of pH 12.5 (14 lines, linewidth 7.9 G, g-value = 2.002) is different from that obtained at pH 8.5, suggesting the formation of two radicals (Fig 5A). In this case, the ESR signal could be detected for about 10 minutes. The effect of the pH on the stability of the orthoquinone was similar to that observed with the catechol. In solutions of pH 8.5 and 12.5, the ortho-quinone gave a comparable ESR signal (Fig 2B and 5B). The free radical signals consisted of 12 and 14 lines, with linewidths of 6.6 and 7.8 G, and g-values of 2.003 and 2.002, respectively. In the ortho-quinone solution of pH 12.5, a more stable free radical was detected after 30 minutes with a different ESR signal (6 lines, linewidth 5.1 G, g-value = 2.007, Fig 6). On incubation of the ortho-quinone at 37°C in phosphate buffer pH 7.4 an ESR signal was immediately observed which decreased during incubation (Fig 3C, D). HPLC-analysis of the incubation mixtures of ortho-quinone and catechol at 37°C in phosphate buffer pH 7.4 or 8.5 showed a similar conversion pattern. At pH 7.4 little conversion was observed, whereas at pH 8.5 conversion was more extensive and products with short retention time were formed and in addition some minor products with longer retention times (Fig 7).

B. Oxidation of the catechol of VP-16-213 by horseradish peroxidase/ hydrogen peroxide and reduction of the ortho-quinone of VP-16-213 by NADPH cytochrome P-450 reductase

Incubation of the catechol of VP-16-213 with the enzymatic one-electron oxidant horseradish peroxidase/ hydrogen peroxide resulted in the formation of a free radical with a hyperfine structure similar to that of the radical formed upon dissolution of the catechol in water at pH 8.5 (Fig 8). HPLC-analysis of the incubation mixture revealed

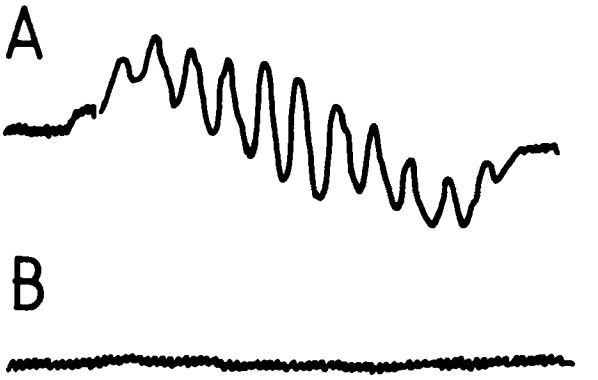

FIGURE 8 A. Free radical obtained on incubation of the catechol of VP-16-213 with HRP/H_2O_2 for 5 min at 37°C and pH 7.4. B. Control incubation without H_2O_2. Instrumental conditions were as described in the legend to Figure 3, except the gain was 2.5×10^5.

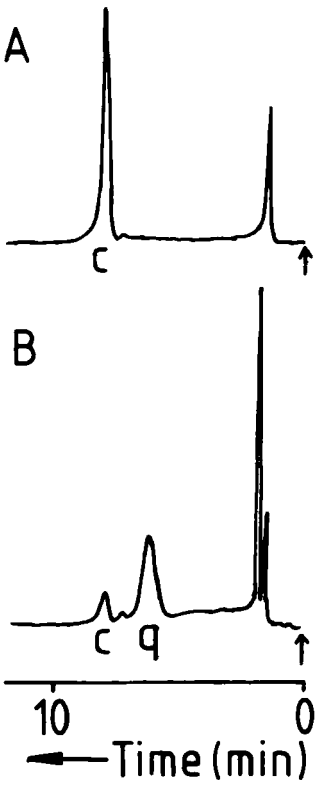

FIGURE 9 HPLC-analysis of the mixture obtained on incubation of the catechol of VP-16-213 with horseradish peroxidase/ hydrogen peroxide at 37°C and pH 7.4 after 0 and 10 min (A and B, respectively). c = catechol, q = quinone.

that after 10 min the catechol was almost completely converted into the ortho-quinone of VP-16-213 and more hydrophilic products (Fig 9). If H_2O_2 was omitted, no free radicals or other products were formed.

Reduction of the ortho-quinone of VP-16-213 by NADPH cytochrome P-450 reductase resulted in the formation of the catechol of VP-16-213, as observed by HPLC-analysis. During incubation of the ortho-quinone of VP-16-213 with NADPH cytochrome P-450 reductase, no free radical was detected. If a solution of the ortho-quinone in DMSO was added to an incubation mixture containing the spin trap POBN, a free radical was detected after about 30 min (Fig 10). The formation of the free radical appeared to be dependent on either NADPH, cytochrome P-450 reductase or DMSO, and was completely inhibited by catalase (Fig 11). The ESR signal increased in intensity with increasing concentration of ortho-quinone and decreased if superoxide dismutase or the iron chelator DTPA were added to the incubation mixture. The ESR signal increased during the incubation. The hyperfine splitting constants of the radical adduct were A_N = 15.8 G and A_H^β = 2.8 G and the g-value was 2.005.

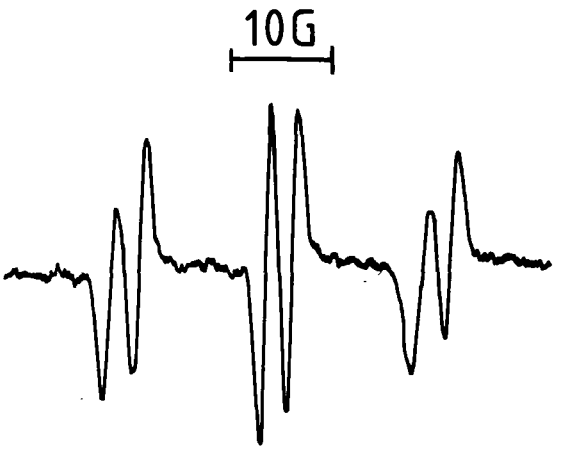

FIGURE 10 POBN- spin adduct formed on incubation of the ortho-quinone of VP-16-213, NADPH, cytochrome P-450 reductase and DMSO. Instrumental conditions were as follows: magnetic field, 3375 G; scan range, 100 G; modulation amplitude, 2.5 G; gain, 8×10^5; scan time, 8 min; time constant, 1 sec.

C. Inactivation of ss ΦX174 DNA by ortho-quinone/ NADPH cytochrome P-450 reductase

Fig 12 shows the survival curves of ss ΦX174 DNA incubated at pH 7.4 with VP-16-213 or the ortho-quinone of VP-16-213 in the presence of NADPH cytochrome P-450 reductase with or without DMSO. Whereas VP-16-213 itself did not inactivate ss ΦX174 DNA, the system ortho-quinone of VP-16-213/ NADPH cytochrome P-450 reductase inactivated ss ΦX174 DNA. The inactivation of ΦX174 DNA by ortho-quinone/ NADPH cytochrome P-450 reductase was inhibited by DMSO. The T_{37}-values – the incubation times resulting in 63% DNA-inactivation – for the incubations with and without DMSO were 1987 and 454 min, respectively.

DISCUSSION

The results of the ESR experiments with alkaline solutions of the catechol and ortho-quinone of VP-16-213 suggest that in both cases the same free radicals were formed. Since the catechol and ortho-quinone of VP-16-213 have a possible common intermediate – the semi-quinone free radical, see Fig 1 – the radical observed may be this semi-quinone. From the linewidth of the spectrum of the free radical obtained from both catechol and ortho-quinone at pH 8.5 – i.e. less than 10 Gauss – it can be concluded that a radical is formed which is delocalized in a π-system. Also, the hyperfine structure shows a pattern similar to that of the phenoxyradical of VP-16-213,[7] suggesting radical formation in the E-ring. The scheme of possible reactions of the catechol and ortho-quinone occurring at alkaline pH and leading to the semi-quinone and secondary radicals is shown in Fig 13. At alkaline pH, the catechol is deprotonated to a dianion, which is easily converted by autooxidation to the semi-quinone radical c, and further to the ortho-quinone. The ortho-quinone is converted by a nucleophilic attack of OH^- at C-6′ to an anion which is converted to the deprotonated 3′, 4′, 6′-trihydroxyderivative of the ortho-quinone. This triol is con-

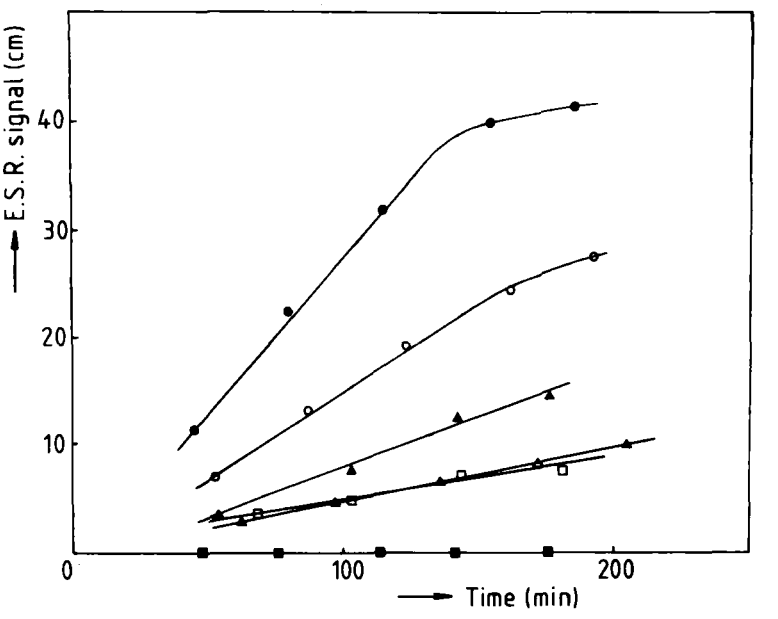

FIGURE 11 The ESR signal of a POBN spin adduct formed on incubation of the ortho-quinone of VP-16-213 (1.3×10^{-4}M, ○ or 2.6×10^{-4}M, ●) with NADPH cytochrome P-450 reductase in the presence of DMSO. □, incubation without ortho-quinone. ■, incubation without DMSO or NADPH or cytochrome P-450 reductase or in the presence of catalase. ▲, incubation in the presence of superoxide dismutase. △, incubation in the presence of DTPA.

verted by autooxidation to a secondary radical g, which can reduce a molecule ortho-quinone to the semi-quinone radical c under formation of a para-quinone. The hydroxylation of an ortho-quinone to a 3′, 4′, 6′-trihydroxyderivative and subsequent oxidation to a para-quinone by another molecule ortho-quinone has also been described for dopaquinone.[16] Similar reactions involving the formation of primary and secondary radicals have been described for 4-methylcatechol and its ortho-quinone.[17] At relatively low alkaline pH, the formation of the semi-quinone c will take place, while at higher pH, the ortho-quinone will be totally converted and only the secondary radical g will be observed. This may explain why for both the catechol and the ortho-quinone variation of the pH results in an alteration of the ESR signal. At pH 8.5 only the semi-quinone radical c is observed, whereas at pH 12.5 a mixture of radicals c and g is observed. In the solutions of the ortho-quinone of pH 12.5 another free radical is formed after about 30 minutes, indicating that at pH 12.5 further conversion takes place of the radical g and the para-quinone. Supportive for the hypothesis that catechol and ortho-quinone form the same products at alkaline pH, is the HPLC-analysis of the incubation mixtures of catechol and ortho-quinone at pH 7.4 and 8.5, which shows the same pattern of products. The ortho-quinone and catechol also show a colour change at pH $\geqslant$ 11, from red to yellow and colourless to yellow, respectively. The reaction scheme shown in Fig 13 is also supported by the observation that an aqueous solution of the ortho-quinone of pH 7.7 can substitute NADPH as electron donor in the reduction of cytochrome c by cytochrome c reductase, indicating that a decomposition product of the ortho-quinone is formed

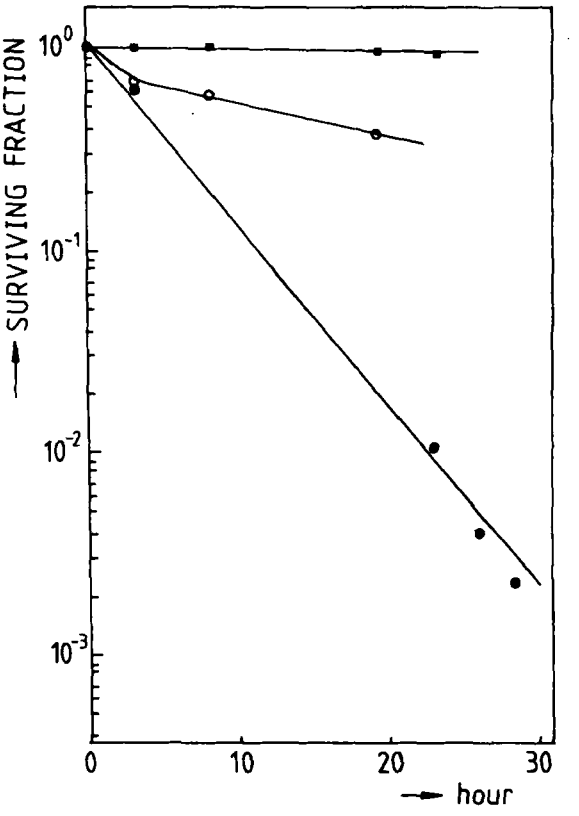

FIGURE 12 Survival curves of single-stranded ΦX174 DNA incubated at pH 7.4 with VP-16-213 (■) and with the ortho-quinone of VP-16-213 and NADPH cytochrome P-450 reductase, in absence (●) or presence (○) of DMSO.

which can be oxidized, e.g. the 3′, 4′, 6′-trihydroxyderivative. The observation that the catechol and ortho-quinone can form a radical at pH 7.4 – which is probably the semi-quinone radical c – might contribute to the elucidation of the mechanism underlying the inactivation of ΦX174 DNA by both compounds.[10]

The one-electron oxidant horseradish peroxidase/ hydrogen peroxide oxidized the catechol of VP-16-213 to the semi-quinone free radical. The radical concentration, however, was very low. The reason for this may be a high oxidation rate for the semi-quinone. The HPLC-analysis showed that already at the start of the incubation with horseradish peroxidase, the catechol had extensively been oxidized to the ortho-quinone, while after 10 min the conversion was complete. The products formed had short retention times.

During reduction of the ortho-quinone of VP-16-213 by NADPH cytochrome P-450 reductase no free radical was detected. This could be due to fast reduction of the ortho-quinone to the catechol and (covalent) binding of the ortho-quinone to the reductase. In the presence of the spin trap POBN a free radical was detected. The effects of incubation conditions on the formation of the POBN spin adduct give information about the nature of the spin adduct. The formation of the spin adduct

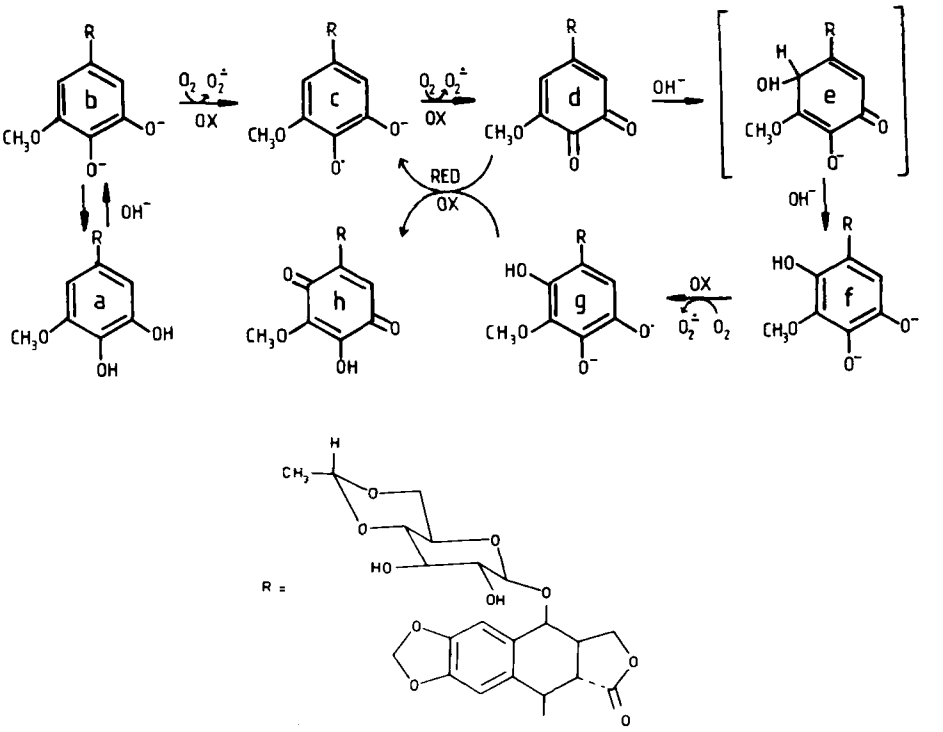

FIGURE 13 Proposed scheme of semi-quinone and secondary radical formation from catechol and ortho-quinone of VP-16-213 in alkaline solution.

increased with ortho-quinone concentration. Remarkably, the spin adduct was also observed in the absence of the ortho-quinone, and its formation appeared to be dependent on the presence of NADPH and cytochrome P-450 reductase, suggesting that the ortho-quinone is involved in redox cycling under formation of superoxide anions during reduction of O_2 by NADPH cytochrome P-450 reductase, which is extensified by the semi-quinone formed by reduction of the ortho-quinone. The superoxide anions may give rise to the formation of hydroxyl radicals by dismutation of O_2- and the Haber-Weiss and Fenton reactions, which has been described by Lai *et al.*[18] This scheme of reactions may also explain the inhibition of the formation of the spin adduct by catalase and the decrease by superoxide dismutase and DTPA. The POBN spin adduct is not the POBN-OH adduct, which has hyperfine splitting constants differing from those of the adduct observed (A_N = 15.0 G and A_H^β = 1.7 G for POBN-OH vs. A_N = 15.8 G and A_H^β = 2.8 G for the spin adduct observed) and is not stable enough to be detected during the long incubation times used, but probably a spin adduct due to a secondary radical formed in a reaction of (OH·) with DMSO.[19] Thus, evidence of formation of oxygen radicals in redox cycling of the ortho-quinone of VP-16-213 during reduction by NADPH cytochrome P-450 reductase was obtained.

The system ortho-quinone/ NADPH cytochrome P-450 reductase inactivated single-stranded ΦX174 DNA and this inactivation was inhibited by DMSO. This indicates that formation of free radicals – e.g. the semi-quinone and/or oxygen

radicals – during reduction of the ortho-quinone plays a role in the process of DNA-inactivation.

In conclusion, evidence was obtained for the formation of the semi-quinone radical from the catechol and ortho-quinone of VP-16-213. Since the semi-quinone may play a role in the inactivation of ΦX174 DNA during reduction of the ortho-quinone by NADPH cytochrome P-450 reductase, either directly or by redox cycling giving formation of oxygen radicals, also these findings suggest that metabolic activation of the E-ring of VP-16-213 plays a role in causing DNA damage. This hypothesis is supported by the recent finding of Teicher *et al.*[20] that VP-16-213 cytotoxicity is greatly enhanced in oxygenated tissue compared to hypoxic tissue. This is in agreement with oxygen-dependent oxidation of VP-16-213 by cytochrome P-450 to the catechol metabolite and redox cycling of the catechol and the ortho-quinone. Since the catechol and ortho-quinone of VP-16-213 both inactivate ΦX174 DNA,[6,10] we will perform studies on the role of semi-quinone and secondary radical formation in the process of DNA inactivation by the catechol and ortho-quinone of VP-16-213.

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