Eur J Cancer Clin Oncol, Vol. 21, No. 10, pp. 1215-1218, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00

Inactivation of ϕX174 DNA by the Ortho-quinone Derivative or its Reduction Product of the Antitumor Agent VP 16-213*

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Abstract—*Biologically active ϕX174 DNA is inactivated by the ortho-quinone derivative of the antitumor agent VP 16-213, but not by VP 16-213 itself, VP 16-213 phenoxy radical or aqueous decomposition product(s) of the ortho-quinone. Reduction of the ortho-quinone by cytochrome P-450 reductase and NADPH results in deactivation of the ortho-quinone towards anti-ϕX174 DNA activity. However, compared with the parent compound VP 16-213, reduction of the ortho-quinone results in substantial damage towards DNA.*

INTRODUCTION

VP 16-213 [4′-demethylepipodophyllotoxin-9-(4,6-*O*-ethylidene-β-D-glucopyranoside), NSC 141540] is an important antineoplastic agent used against a variety of tumors [1]. Its precise mechanism of action is unknown. Loike and Horwitz [2] studied its effect on HeLa cell DNA and concluded that VP 16-213 causes DNA single-strand breaks. More recently, Wozniak and Ross [3] reported that cytotoxicity of VP 16-213 in L1210 cells is probably caused by DNA damage.

In our study on the metabolism of VP 16-213 we observed that the major metabolite formed in the rat is the hydroxy acid derivative, produced after opening of the lactone-ring [4]. However, this hydroxy acid metabolite did not show cytotoxic effects [5]. We recently published in this journal that cytochrome P-450-mediated covalent binding of VP 16-213 occurs to rat liver microsomal proteins, and that the dimethoxyphenol ring of VP 16-213 (the pendant ring) is probably involved in the binding process [6]. The latter observation and the following two observations from the literature prompted us to investigate possible chemical and biological conversions of the dimethoxyphenol ring of VP 16-213: (a) the effect on DNA appears to require the phenol group of the pendant ring [2]; and (b) isolated purified DNA is not broken down by the parent drug [2]. One of the active metabolites of VP 16-213 may be the phenoxy radical, which can be formed by one-electron oxidation of VP 16-213 [7, 8]. Recently we observed that incubation of VP 16-213 with cytochrome P-450, cytochrome P-450 reductase and NADPH (oxygenation) or with cytochrome P-450 and cumene hydroperoxide (peroxygenation) resulted in *O*-demethylation of VP 16-213 [9]. The product of *O*-demethylation — the ortho-dihydroxy derivative of VP 16-213 (the catechol) — in turn may be converted by oxidation to the ortho-quinone of VP 16-213. The possible conversions of the dimethoxyphenol ring of VP 16-213 are summarized in Fig. 1.

In order to obtain information about the effects of metabolites of VP 16-213 on the biological activity of DNA, we investigated anti-DNA activity of VP 16-213, the phenoxy radical, the ortho-quinone and reduction products of the ortho-quinone. The test system we used was biologically active ss ϕX174 DNA.

Accepted 16 April 1985.

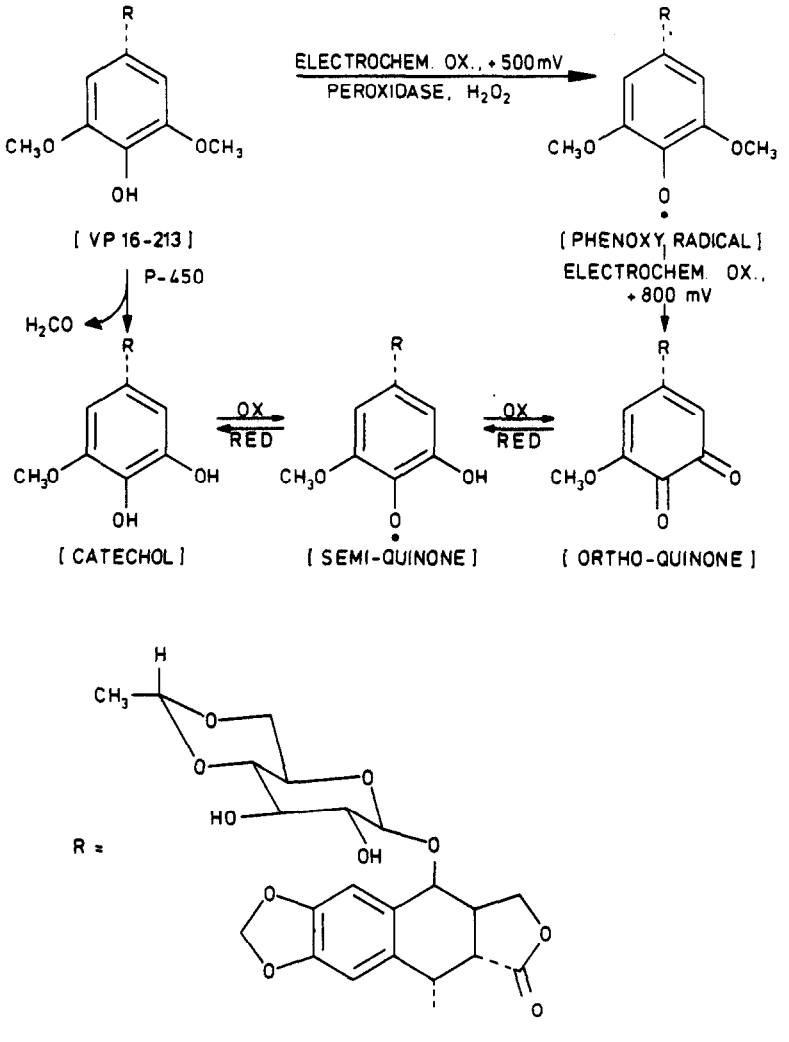

Fig. 1. Possible conversions of the dimethoxyphenol ring of VP 16-213.

MATERIALS AND METHODS

DNA and chemicals

Details concerning the preparation of single-stranded DNA of the bacteriophage ϕX174 DNA and determination of its biological activity (spheroplast test) have been described before [10, 11]. Briefly, 0.1 ml of 25 $\times$ 10^{-9} mol/dm^3 single-stranded ϕX174 DNA was mixed with an equal volume of spheroplast of *Escherichia coli* K12. After 10 min at 20°C, 0.8 ml of LBM [Luria Broth with 10% (w/v) sucrose/0.1% (w/v) glucose/0.2% (w/v) $MgCl_2$] medium was added and incubation was continued (37°C) for an additional 2 hr. After this 4 ml of cold distilled water was added and the bacteriophage titers were determined by plating using *E. coli C* as the indicator bacterium.

VP 16-213 was a gift from the Bristol Myers Company (Syracuse, NY, U.S.A.). The VP 16-213 phenoxy radical was obtained by electrochemical oxidation of VP 16-213 at +500 mV using an electrochemical cell with Pt as the working and auxiliary electrode and Ag/AgCl as the reference electrode. The formation of the phenoxy radical was analyzed by electron spin resonance spectrometry on a Varian E-3 spectrometer [8]. The ortho-quinone of VP 16-213 was synthesized by controlled potential electrolysis of VP 16-213 at a Pt gauze electrode [12].

Cytochrome P-450 reductase was purified from phenobarbital-induced rat liver microsomal preparations according to the method of Guengerich and Martin [13]. All other chemicals were of analytical grade. Spectrophotometry was performed on a Beckman model 35 spectrophotometer.

Incubations with ss ϕX174 DNA

Prior to the spheroplast test, solutions of single-stranded ϕX174 DNA (5×10^{-7} mol/dm^3 nucleotides) and 5×10^{-2} mol/dm^3 potassium phosphate pH 7.4 were incubated at 37°C with VP 16-213 (170×10^{-6} mol/dm^3), the VP 16-213 phenoxy radical (15×10^{-6} mol/dm^3) and the ortho-quinone (175×10^{-6} mol/dm^3) alone and in the presence of cytochrome P-450 reductase (0.31

units) and NADPH (10^{-5} mol/dm^3). Also, incubations were performed of DNA and 5 × 10^{-2} mol/dm^3 potassium phosphate, pH 4.0, with the ortho-quinone (175 × 10^{-6} mol/dm^3). At several intervals of incubation, samples were taken for the spheroplast test.

RESULTS

Incubation of ss ϕX174 DNA with VP 16-213 or the phenoxy radical of VP 16-213 did not result in a decrease of DNA activity.

Before studying the effect of the ortho-quinone of VP 16-213 on the activity of ss ϕX174 DNA, we investigated its stability by spectrophotometry. Incubation in buffer at pH 7.4 and 4.0 at 37°C revealed that the ortho-quinone is stable at pH 4.0, while it slowly decomposes upon incubation at pH 7.4. Therefore, incubations of the ortho-quinone with DNA were performed at pH 7.4 and pH 4.0. Figure 2 shows survival curves for ss ϕX174 DNA incubated at pH 7.4 with the ortho-quinone of VP 16-213 alone and in the presence of cytochrome P-450 reductase and NADPH, and at pH 4.0 with the ortho-quinone of VP 16-213 alone.

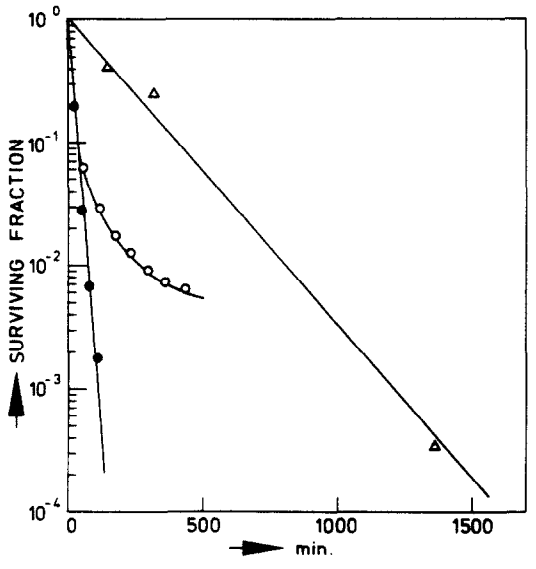

Fig. 2. *Survival curves for single-stranded* ϕX174 *DNA* (5 × 10^{-7} mol/dm^3) *dissolved in* 5 × 10^{-2} mol/dm^3 *phosphate buffer* (*pH* 7.4) *with* 175 × 10^{-6} mol/dm^3 *ortho-quinone alone* (○) *and in the presence of* 0.31 *unit cytochrome P-450 reductase and* 10^{-3} mol/dm^3 *NADPH* (△), *and dissolved in* 5 × 10^{-2} mol dm^3 *phosphate buffer* (*pH* 4.0) *with* 175 × 10^{-6} mol/dm^3 *ortho-quinone* (●).

The survival curve for DNA incubated with the ortho-quinone at pH 4.0 shows a steep decline, while the survival curve for DNA incubated with the ortho-quinone at pH 7.4 starts with the same decline, but then strongly deflects. The survival curve for DNA incubated with the ortho-quinone, cytochrome P-450 reductase and NADPH at pH 7.4 starts with a less steep decline than the curves for DNA with the ortho-quinone alone. In contrast to the curve for DNA with the ortho-quinone alone at pH 7.4, this curve does not deflect.

DISCUSSION

The inability of VP 16-213 to cause lethal damage to ss ϕX174 DNA confirms the necessity of activation of the drug for DNA-interaction. The lack of effect of the phenoxy radical of VP 16-213 is in accordance with the described inactivity towards DNA of phenoxy radicals, produced by gamma radiolysis [11].

From the survival curves for DNA incubated with the ortho-quinone at pH 4.0 and 7.4, we conclude that the ortho-quinone causes an extensive inactivation of DNA, while aqueous decomposition products of the ortho-quinone formed at pH 7.4 most probably do not inactivate DNA. Reduction of the ortho-quinone by cytochrome P-450 and NADPH at pH 7.4 leads to lesser inactivation of the DNA than in the case of the ortho-quinone itself. However, since the survival curve does not deflect, the inactivation of DNA during reduction of the ortho-quinone cannot be attributed to the presence of non-reduced ortho-quinone. The conclusion is that the reduction product of the ortho-quinone can also inactivate the DNA.

From the slopes of the survival curves for DNA with the ortho-quinone at pH 4.0 and for DNA with the ortho-quinone in the presence of cytochrome P-450 reductase and NADPH at pH 7.4, the following T_{37} values (T_{37} = incubation time resulting in 63% DNA-inactivation), corrected for control values, were calculated: 24 and 384 min, respectively.

In conclusion, the ortho-quinone of VP 16-213 and its reduction product cause extensive inactivation of ss ϕX174 DNA, and probably contribute to the anti-DNA activity of VP 16-213. This is the first time it has been shown that a change in the phenolic structure of VP 16-213 leads to a compound which inactivates DNA.

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