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EFFECTS OF THE ANTITUMOR DRUGS 3-NITROBENZOTHIAZOLO {3,2-*a*}QUINOLINIUM AND FAGARONINE ON NUCLEIC ACID AND PROTEIN SYNTHESIS*

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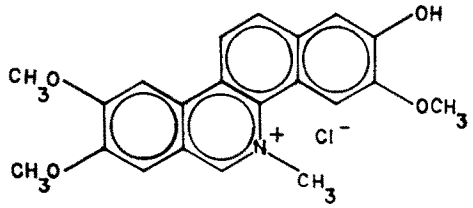
Abstract—3-Nitrobenzothiazolo{3,2-*a*}quinolinium perchlorate (NBQ) has been shown to be active against *in vivo* experimental tumors of P388 and Ehrlich ascites cells. Furthermore, it has been established that NBQ binds to DNA by intercalation. In this work we describe its effects on DNA, RNA and protein syntheses both in KB cells and in cell-free synthesizing systems. Fagaronine, an alkaloid structurally related to NBQ, was studied also in an attempt to establish the basis for future studies on structure–activity relationships. Both NBQ and fagaronine inhibited DNA, RNA and protein syntheses in KB cells, with essentially equal effectiveness. Exposure of KB cells to NBQ for 2 hr caused irreversible inhibition of DNA, RNA and protein syntheses. Studies in cell-free systems showed that NBQ strongly inhibited *Escherichia coli* DNA polymerase I, whereas RNA polymerase activities were moderately affected. Furthermore, both drugs inhibited protein synthesis in cell-free systems derived from rabbit reticulocytes and *Saccharomyces cerevisiae*. Our results indicate that NBQ and fagaronine exert their cytotoxic activity by at least two independent mechanisms: inhibition of DNA activity by binding to this molecule, and inhibition of protein synthesis probably by interacting with the ribosomal system.

The benzophenanthridine alkaloids fagaronine [1] (Fig. 1a) and nitidine [2] have shown antitumor activity against P388 and L1210 murine leukemias. Studies on the mechanism of action of both alkaloids suggested that they inhibit reverse transcriptase activity by interacting with the A:T template primer [3, 4]. 3-Nitrobenzothiazolo[3,2-*a*]quinolinium (NBQ; Fig. 1b) was synthesized by Cox *et al.* [5] as part of a program aimed at obtaining structural analogues of fagaronine and nitidine. NBQ has been found to be active against KB and Hela cells in culture and against P388 leukemia and Ehrlich ascites tumor *in vivo* [5]. Furthermore, our previous studies on the biophysical interaction of NBQ with the DNA molecule have shown that it binds to DNA via an intercalation mechanism [6]. These studies have revealed that the interaction of NBQ with A:T-rich DNA is stronger than the interaction of NBQ with G:C-rich DNA, thus suggesting that NBQ displays a preference for A:T base pairs [6].

The present study was undertaken in an attempt to elucidate the mechanism(s) of action of NBQ and fagaronine.

MATERIALS AND METHODS

Drugs. NBQ was a gift of Dr. O. Cox (Department of Chemistry, University of Puerto Rico, Río Piedras, Puerto Rico). Fagaronine (NCS 157995) was provided by Dr. J. D. Douros (Natural Products Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD). Narciclasine was isolated from bulbs of narcissus [7]. Solutions of drugs were made fresh before use. Drugs were dissolved in 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) buffer (pH 7.0) with dimethyl sulfoxide (DMSO) at a final concentration of 0.8%. The molar concentrations of NBQ and fagaronine



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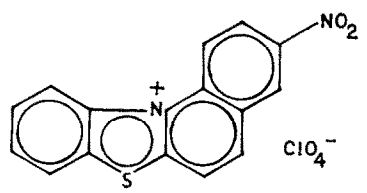

1b

Fig. 1. Structural formulae of fagaronine (1a) and 3-nitrobenzothiazolo[3,2-*a*]quinolinium (1b).

* A preliminary report of this work was presented before the Federation of European Biochemical Societies (15th FEBS Meeting; Abstr. S-01 TU-008).

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were determined spectrophotometrically by measuring the absorbance at 273 ($\varepsilon = 26{,}083$) and 328 ($\varepsilon = 28{,}093$) nm, respectively, in a Beckman B-25 UV.VIS spectrophotometer.

Reagents. Escherichia coli RNA polymerase (5,000 units/ml), *Escherichia coli* DNA polymerase I (35,000 units/ml) and poly (deoxyadenylate-deoxythymidylate) were the products of P.L. Biochemicals, Milwaukee, WI. Creatine phosphokinase, yeast tRNA, polyuridylic acid, creatine phosphate, bovine albumin, deoxyribonucleotides and ribonucleotides were purchased from the Sigma Chemical Co., St. Louis, MO. [^{3}H]Thymidine (51 Ci/mmole), [^{3}H] uridine (4.4 Ci/mmole), [^{3}H]leucine (130 Ci/mmole), [^{14}C]phenylalanine (509 mCi/mmole), [^{3}H] thymidine 5′-triphosphate (10 Ci/mmole), and [^{3}H] uridine 5′-triphosphate (10 Ci/mmole) were purchased from Amersham Radiochemicals, Arlington Heights, IL.

Cells and growth conditions. KB and Ehrlich ascites cells were used throughout this work. KB cells were maintained as a monolayer culture in "Difco TC-Minimal Medium Eagle, Dried" and in suspension culture in "Difco TC-Minimal Medium Eagle, Spinner Modified, Dried" (Medium S). Both media were supplemented with 10% (v/v) fetal calf serum and antibiotics. Ehrlich ascites tumor cells were maintained and grown in male CF-1 mice. Tumor transfer was performed every 6–8 days.

Preparation of ribosomes. High salt washed ribosomes from *Saccharomyces cerevisiae* cells, strain Y166, were prepared as described previously [8, 9].

Synthesis of macromolecules. Most of the experiments were performed with KB cells. Exponentially growing cells were detached from culture vessels by treatment with 0.25% trypsin and suspended in medium S at 4×10^5 viable cells/ml assayed by the trypan blue exclusion test as described by Phillips [10]. Ehrlich ascites tumor cells were collected 7–8 days after transplantation and freed from erythrocytes by washing twice with 0.85% NaCl solution and recovered by mild centrifugation. The washed cells were then suspended in a volume of medium S to give a viable cell density of 4×10^5 cells/ml assayed by the trypan blue dye exclusion test as described by Phillips [10].

Aliquots (0.6 ml) of the cell suspension were incubated with the indicated amount of drugs. Control cells with no drug were included in each assay. Then, 1 μCi/ml of [^{3}H]thymidine, or 1 μCi/ml of [^{3}H]uridine, or 2 μCi/ml of [^{3}H]leucine was added to triplicate cultures. In those flasks with the labeled amino acids, the medium was removed and replaced with phosphate-buffered saline (PBS) at 37° prior to adding the labeled amino acids.

After incubation for 90 min at 37°, the assay solution was made 10% (w/v) with trichloroacetic acid (TCA) and 1% (w/v) sodium pyrophosphate. Braña *et al.* [11] showed that over a 90-min incubation the incorporation followed a linear time course with Ehrlich ascites tumor cells. Similar results were found in our laboratory with EAT and KB cells (results not shown; $r = 0.98$; 0.97). To measure protein synthesis, samples were boiled for 15 min at 90°. Samples were chilled to 0° and allowed to stand at least 30 min on ice. The resulting precipitates were collected on glass fiber filters and washed with 10% TCA and ethanol and dried. For DNA and RNA extraction, the dried discs were extracted with 0.5 N perchloric acid at 70° for 1 hr. Radioactivity was counted in Ready-Solv MP (Beckman).

For time course studies, 10-ml aliquots of cell suspension (4×10^5 cells/ml) were incubated with the appropriate labeled metabolite at 37°. The drugs were added after 15 min of incubation. At different times 0.5-ml samples were taken and processed for measurements of radioactivity as indicated above.

The reversibility of the effect of the drugs on nucleic acid and protein synthesis in KB cells was assayed essentially according to the method reported by Huang and Grollman [12].

DNA polymerase activity. DNA polymerase I from *E. coli* was assayed essentially by the method described by Setlow [13]. Incubations were carried out in a final reaction volume of 25 μl containing: 0.01 units of enzyme; 0.18 A_{260} units of poly (dA-dT); 20 μmoles potassium phosphate buffer (pH 7.4); 2 μmoles $MgCl_2$; 10 mmoles dATP and 7 μM [^{3}H]dTTP (10 Ci/mmole). Mixtures were incubated at 16° for 20 min. The reaction was stopped by adding 2 ml of cold 0.6 M perchloric acid with 5 mM sodium pyrophosphate. Samples were chilled at 4° for 15 min, and the precipitates were collected on Whatman GF/C, washed three times with 0.4 M perchloric acid and twice with 95% ethanol. Filters were dried and radioactivity was measured as previously described.

RNA polymerase activity. Assays were performed according to the procedure of Maitra and Hurwitz [14]. The reaction mixture contained in a final volume of 50 μl: 25 μmoles potassium phosphate buffer (pH 7.4); 2 μmoles mercaptoethanol; 0.5 μmole spermine; 2 μmoles $MgCl_2$; 80 μmoles ATP; 50 nmoles [^{3}H]UTP (10 Ci/mmole); 2 units of enzyme; and 1 unit of poly (dA-dT). The reaction was incubated at 37° for 10 min and stopped by adding 2 ml of cold 20% TCA with 1% pyrophosphate and collected on Whatman GC/F filters. Samples were processed for radioactivity measurement as indicated above.

Cell-free protein-synthesizing system. Drug effects on cell-free protein synthesis were tested in a rabbit reticulocyte system (BRL Inc., MD) The total volume of reaction mixture was 30 μl, containing 3 μl of reaction mixture [250 μM HEPES (pH 7.2), 400 μM potassium chloride, 10 mM creatine phosphate and 500 μM of each amino acids except leucine]; 3 μl of [^{3}H]leucine (130 Ci/mmole); 1.3 μl of 2.0 M potassium acetate; 10 μl of rabbit reticulocyte lysate (3 × concentrated) containing 3.5 mM magnesium chloride, 0.05 mM EDTA, 25 μM potassium chloride, 0.5 mM dithiothreitol, 25 μM hemin, 50 μg/ml of creatine kinase, 1 μM calcium chloride, 2 mM ethyleneglycolbis(amino - ethylether)tetra - acetate (EGTA), and 70 mM sodium chloride, 0.04 μg mRNA and 3 μl of drugs at the indicated concentrations. H_2O was used to adjust the final volume to 30 μl. Mixtures were incubated at 30° for 1 hr. Incorporation of [^{3}H]leucine proved to be linear with increased pulse time up to 1 hr (data not shown).

The reaction was stopped by adding 20 μl of bovine albumin (1 mg/ml) and 2 ml of cold 20% TCA.

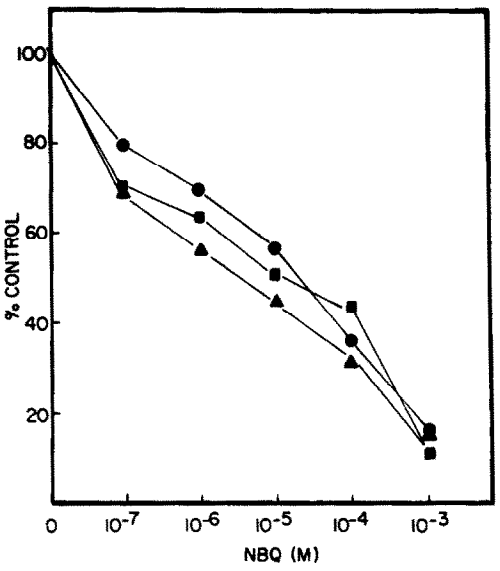

Fig. 2. Effects of different concentrations of NBQ on DNA, RNA, and protein syntheses in KB cells. Drug was added at the indicated final concentrations to 0.6 ml KB cells (4×10^5 cells/ml) suspended in medium S containing the appropriate precursor: [^{3}H]thymidine (1 μCi/ml), [^{3}H]-uridine (1 μCi/ml) and [^{3}H]leucine (1 μCi/ml) to measure DNA (●), RNA (▲), and protein (■) synthesis respectively. Mixtures were incubated at 37° for 90 min, the reaction was terminated, and the rates of precursor incorporation were determined as described under Materials and Methods. The percentage incorporation shown is expressed relative to cell cultures to which no inhibitor was added. Data points are the average of at least four experiments.

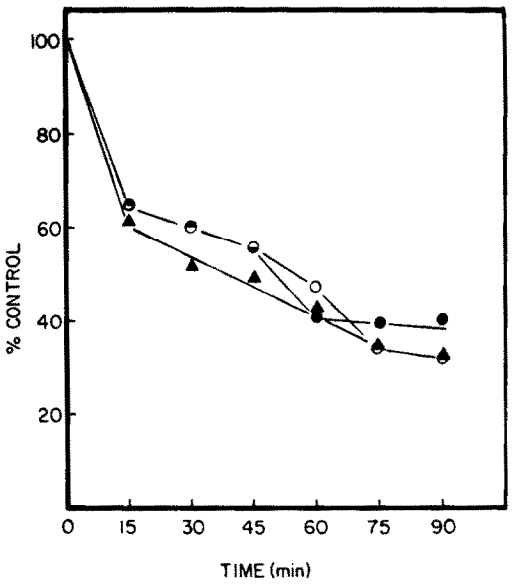

Fig. 3. Time course of inhibition of macromolecule synthesis induced by NBQ in KB cells . KB cells (4×10^5 cells/ml), suspended in medium S, were divided into two aliquots of 10 ml each, and NBQ was added to one of the aliquots at a final concentration of 2×10^{-4} M. Then, 1 μCi/ml of [^{3}H]thymidine, or 1 μCi/ml of [^{3}H]uridine, or 1 μCi/ml of [^{3}H]leucine was added to both culture vessels to measure DNA (○), RNA (▲), and protein (●) synthesis respectively. The cell suspensions with the appropriate precursors were incubated at 37° and 0.5-ml aliquots were taken at the indicated times. Then, the rates of DNA, RNA and protein syntheses were determined as described under Materials and Methods. Data points are the average of three experiments. Values in cpm for 100% in the various experiments in this figure were as follows: DNA, 28,400–31,300; RNA, 7,500–13,000; and protein 7,900–11,300.

Samples were heated at 90° for 10 min, chilled for 15 min, and collected on Whatman GF/C filters. The samples were counted in a scintillation counter as described previously.

The effects of NBQ and fagaronine on poly (U)-directed [^{14}]phenylalanine incorporation into TCA precipitable polypeptides were studied in a ribosomal system derived from *S. cerevisiae*, strain Y166, according to methods already published [8, 9].

Table 1. IC_{50} Values for inhibition of macromolecular synthesis by NBQ and fagaronine

Drug	Cell Line	IC_{50} (μM) DNA	RNA	Protein
NBQ	KB	13.5	3.0	8.3
	EAT	24.8	4.2	20.5
Fagaronine	KB	38.4	11.6	16.0

Drugs were added to 0.6 ml of cells (4×10^5 cells/ml) with the appropriate labeled metabolite and incubated for 90 min at 37°. Percentage of inhibition was calculated from the specific activity of incorporation (cpm/10^6 cells) of drug-treated as compared to those of the control. IC_{50} Values were calculated by plotting the logarithmic drug concentrations against the percentage of inhibition of macromolecule synthesis.

RESULTS

Inhibition of macromolecule synthesis. The effects of NBQ on DNA, RNA and protein syntheses were measured in KB and Ehrlich ascites tumor cells following the incorporation of radioactive precursors into the acid-insoluble fraction. Figure 2 is illustrative of the effects of NBQ on macromolecule synthesis after a 90-min exposure to various concentrations of the drug. A dose–effect relationship was observed, and DNA, RNA and protein syntheses were inhibited with almost equal effectiveness.

Concentrations of NBQ and fagaronine producing 50% inhibition in this system were estimated from data similar to that shown in Fig. 2 and are given in Table 1. KB cells appeared to be slightly more sensitive to the effects of NBQ. NBQ gave a 50% inhibition of RNA synthesis at 3–4 μM concentration, whereas a 5-fold increase in concentration was needed to inhibit DNA and protein syntheses.

The rate of inhibition of macromolecule synthesis in KB cells induced by fagaronine was remarkably similar to that exerted by NBQ. Again, RNA syntheses was inhibited by (IC_{50} = 11.6 μM) more effectively than DNA and protein syntheses.

A time course of the effect of NBQ on [^{3}H]uridine,

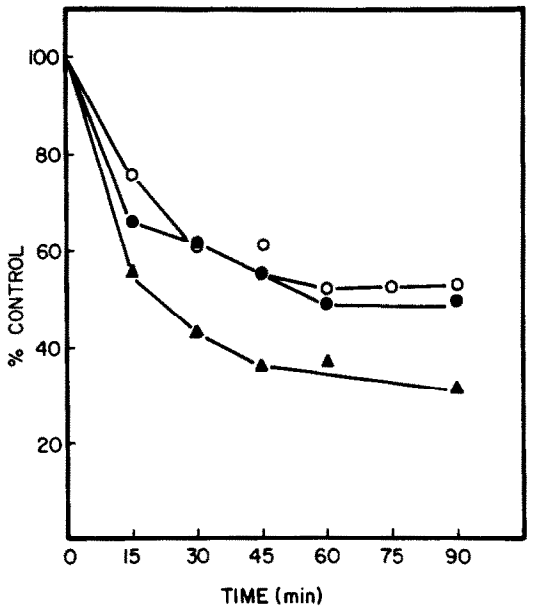

Fig. 4. Time course of inhibition of macromolecule synthesis induced by fagaronine in KB cells. KB cells (4×10^5 cells/ml), suspended medium S, were divided into two aliquots of 10 ml each, and NBQ was added to one of the aliquots at a final concentrations of 2×10^{-4} M. Then, 1 μCi/ml of [^{3}H]thymidine, or 1 μCi/ml of [^{3}H]uridine, or 1 μCi/ml of [^{3}H]leucine was added to both culture vessels to measure DNA (○), RNA (▲), and protein (●) synthesis respectively. The cell suspensions with the appropriate precursors were incubated at 37° and 0.5-ml aliquots were taken at the indicated times. Then, the rates of DNA, RNA and protein syntheses were determined as described under Materials and Methods. Data points are the average of three experiments. Values in cpm for 100% in the various experiments in this figure were as follows: DNA, 28,4000–31,300; RNA, 7,500–13,400; and protein 7.900–11,300.

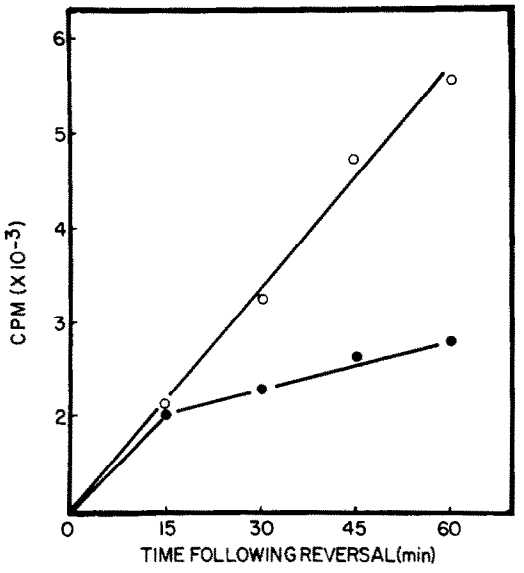

Fig. 5. Reversal of inhibition of DNA synthesis induced by NBQ in KB cells. KB cells (5×10^5 cells/ml), suspended in medium S, were incubated for 2 hr in the presence of 2×10^{-4} M NBQ. The control culture (○——○) contained no antibiotics. All cultures, including the control, were washed four times with Earle's solution at 37° and resuspended in fresh medium; 1 μCi/ml of [^{3}H]thymidine was added to each culture, and the rate of DNA synthesis was measured as described under Materials and Methods. Data points are the average of three experiments.

[^{3}H]thymidine or [^{3}H]leucine incorporation in acid-precipitable material is shown in Fig. 3. The concentration used in this experiment was determined from previous experiments as the one that would inhibit macromolecule synthesis by 75% for at least 1 hr. At 200 μM NBQ, the effect was evident within 15 min of treatment; DNA, RNA and protein syntheses were inhibited by 36, 39 and 34% of the control. The inhibition increased with contact time arriving at a plateau after 75 min of exposure and continued in that range for at least 4 hr (results not shown). Parallel assays with fagaronine (Fig. 4) showed that the alkaloid follows a similar pattern and, after 15 min of exposure, it produced a 45% inhibition of RNA synthesis, 34% inhibition of protein synthesis and 21% inhibition of DNA synthesis. Inhibition of RNA synthesis became more pronounced as compared to that produced by NBQ at the same concentration.

Reversibility of action. After 2 hr of contact with KB cells, NBQ (200 μM) was removed by centrifugation, cells were resuspended in fresh medium, and the residual effect was measured following the incorporation of radiolabeled precursors (Figs. 5–7). Control cultures incorporated precursors into

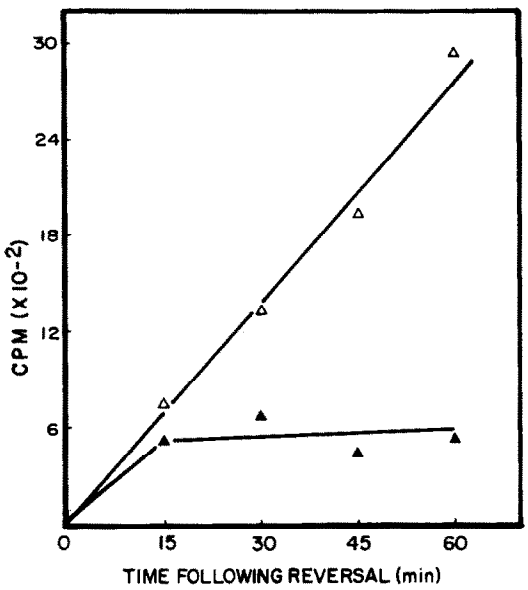

Fig. 6. Reversal of inhibition of RNA synthesis induced by NBQ in KB cells. KB cells (5×10^5 cells/ml), suspended in medium S, were incubated for 2 hr in the presence of 2×10^{-4} M NBQ. The control culture (△——△) contained no antibiotics. All cultures, including the control, were washed four times with Earle's solution at 37° and resuspended in fresh medium; 1 μCi/ml of [^{3}H]uridine was added to each culture, and the rate of RNA synthesis was measured as described under Materials and Methods. Data points are the average of three experiments.

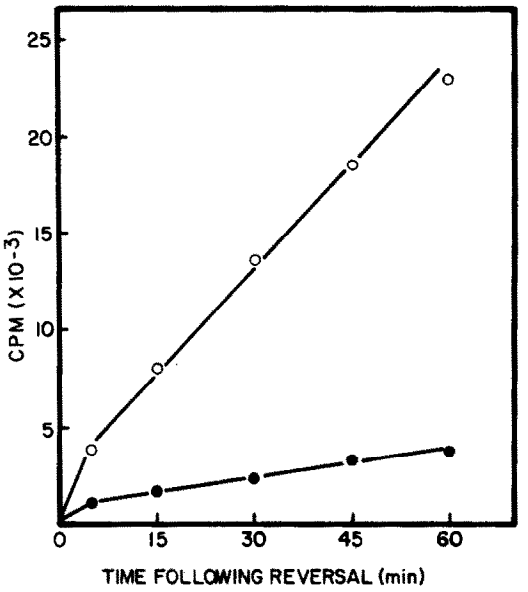

Fig. 7. Reversal of inhibition of protein synthesis induced by NBQ in KB cells. KB cells (5×10^5 cells/ml), suspended in medium S, were incubated for 2 hr in the presence of 2×10^{-4} M NBQ. The control culture (O——O) contained no antibiotics. All cultures, including the control, were washed four times with Earle's solution at 37° and resuspended in fresh medium; 1 μCi/ml of [^{3}H]leucine was added to each culture, and the rate of protein synthesis was measured as described under Materials and Methods. Data points are the average of three experiments.

macromolecules rapidly; meanwhile, cells previously treated with NBQ remained inhibited 4 hr after the NBQ had been removed. The effect on incorporation of amino acids into protein was the most potent and irreversible under the conditions used. There was partial recovery of precursor incorporation into DNA and RNA up to 15 min after removal of NBQ. However, on continued incubation a marked decline in uridine and thymidine incorporation was observed. After 60 min of incubation, uridine incorporation was inhibited by 70% and thymidine incorporation was inhibited by 61%.

Effects on DNA polymerase I and RNA polymerase of E. coli. Since NBQ has been shown to bind DNA by intercalation [6], we studied the effects

Table 2. Inhibition of *E. coli* DNA polymerase I by NBQ and fagaronine

Concentration (M)	Inhibition (%)	
	NBQ	Fagaronine
10^{-6}	4	9
10^{-5}	36	47
10^{-4}	74	82
10^{-3}	92	98

The activity of *E. coli* DNA polymerase I was determined as described in the text. The degree of inhibition was determined by comparison to the control. Control enzyme activity was 13.5 mmoles of dTMP incorporated/20 min/ml.

Table 3. Inhibition of *E. coli* RNA polymerase activity by NBQ and fagaronine

Concentration (M)	Inhibition (%)	
	NBQ	Fagaronine
10^{-7}	19	14
10^{-6}	27	16
10^{-5}	50	44
10^{-4}	54	50

RNA polymerase activity was estimated according to the method described in the text. The degree of inhibition was determined by comparison to the control. Control enzyme activity was 83.2 pmoles UMP incorporated.

of NBQ and fagaronine on the function of DNA as a template primer. Previous studies by Sethi and coworkers [3, 4] have shown that fagaronine strongly inhibits DNA polymerase activity by blocking its action as a template primer. The results, summarized in Table 2, indicate that at 1 mM both compounds inhibited approximately 90% of the DNA polymerase activity. Meanwhile, when the concentration was reduced 100-fold, fagaronine (47%) showed higher activity than NBQ (36%). Increasing the concentration of the template 3-fold reduced the inhibitory effect of 10 μM NBQ from 36 to 23% of the control values.

Table 3 shows the effects of NBQ and fagaronine on RNA polymerase activity. Enzyme activity was inhibited in a dose-related fashion. Inhibition of RNA polymerase activity by both compounds was less pronounced than the effect on DNA polymerase I activity. Fifty percent of the polymerase activity was reduced by NBQ and fagaronine at 56 and 86 μM respectively.

Effects on cell-free translation systems. Since inhibition of protein synthesis appeared to be a major effect of NBQ or fagaronine, the effects of both compounds on protein synthesis in a cell-free system were further evaluated. Table 4 shows the effects of NBQ and fagaronine on globin synthesis in a ribosomal system derived from rabbit reticulocytes. Narciclasine, a specific inhibitor of ribosomal protein synthesis, was used as a positive control. Under the experimental conditions used, NBQ and fagaronine at 100 μM blocked protein synthesis by 43% and 50%, respectively, in rabbit reticulocytes. We also studied the effects of fagaronine and NBQ on the polyuridine-directed synthesis of [^{14}C]polyphenyl-

Table 4. Inhibition of globin synthesis in a cell-free translation system derived from rabbit reticulocytes

Inhibitor	Inhibition (%)	
	10^{-4} M	10^{-3} M
NBQ	43	70
Fagaronine	50	77
Narciclasine	65	91

Globin synthesis in rabbit reticulocytes was measured as described under Materials and Methods. Control enzyme activity was 12.5 pmoles of [^{3}H] leucine incorporated. Results are the average of duplicate experiments.

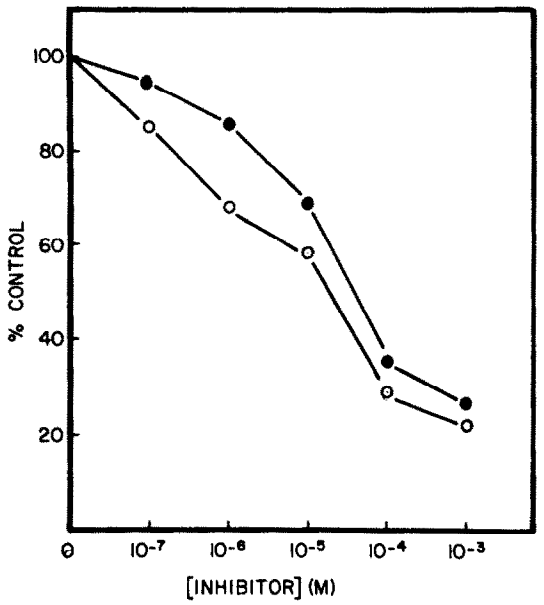

Fig. 8. Effects of NBQ and fagaronine on [^{14}C]phenylalanine, incorporation directed by poly (U) in *S. cerevisiae* ribosomes. The reaction mixture contained in 100 μl: 50 mM Tris–HCl (pH 7.4); 12.5 mM $MgCl_2$; 80 mM KCl; 10 mM mercaptoethanol; 4 mM creatine phosphate; 1 mM ATP; 5 mM GTP; 40 μg/ml of creatine phosphokinase; 250 μg/ml of yeast tRNA; 30 μg/ml of poly (U); 60 μCi [^{14}C]phenylalanine; optimal concentration of supernatant fraction; and 0.1 mg of yeast ribosomes. Mixtures were incubated at 30° for 30 min and then polypeptide synthesis was stopped by adding 20 μl bovine albumin (1 mg/ml) and 2 ml of 10% TCA. Samples were processed for radioactivity determination. Results are the average of two experiments, and 52.5 pmoles of [^{14}C]phenylalanine were incorporated in the control. Key: percentage of [^{14}C]phenylalanine incorporation in the presence of fagaronine (—●—) or NBQ (—○—).

alanine in a yeast ribosomal system, in order to investigate the effects of these compounds on the elongation steps of protein synthesis. Figure 8 shows that NBQ and fagaronine inhibited polyphenylalanine synthesis. At 100 μM, NBQ caused 72% inhibition, whereas fagaronine caused 65% inhibition.

DISCUSSION

The present investigation provides strong evidence that NBQ and fagaronine exert their cytotoxic activities by at least two independent mechanisms. These mechanisms involve the inhibition of nucleic acid activity by interaction with the DNA molecule and the inhibition of protein synthesis by interaction with the protein-synthesizing apparatus. This behavior is not surprising since it has been reported previously that many drugs exert cytotoxic activity by interacting with a number of cellular target sites [15–17].

NBQ intercalates with the DNA molecule to form a reversible complex [6]. Moreover, fagaronine stabilizes calf thymus DNA against heat denaturation [6] and induces an increase in the viscosity of native DNA, which suggests that fagaronine interacts with the DNA molecule probably by intercalation (results not shown).

We have found that NBQ inhibited the incorporation of thymidine, uridine and leucine into KB and Ehrlich ascites tumor cells. Of particular note was that the inhibitions were strikingly similar, and there was not a definite strong preferential effect on the nucleic acid syntheis versus the protein synthesis. The concentrations required to achieve 50% inhibition of DNA, RNA and protein syntheses were 14–25, 3–4 and 18–20 μM respectively (Table 1). These concentrations are higher than those required for inhibition of cell growth (ED_{50} = 0.6 μg/ml; Ref. 5). However, cell growth inhibitory assay results were obtained after 72 hr of incubation of cells with NBQ, whereas inhibition of macromolecule synthesis results were obtained after 2 hr of incubation which could account for this difference.

Fagaronine showed a similar pattern of inhibition to that observed with NBQ. Nevertheless, fagaronine showed a marked effect on RNA synthesis (Fig. 4) which suggests that the alkaloid may act preferentially at the level of transcription.

In our study, inhibition of DNA and RNA synthesis was partially reversible up until 15 min after removal of NBQ. Then a rapid inhibition followed and continued to increase with time. This phenomenon could be ascribed to the degradation of the DNA molecule. Ross *et al.* [18–20] have presented data showing that a common feature of intercalators appears to be DNA single-strand scission and associated DNA-proteins cross-links.

In contrast, the effect of NBQ on protein synthesis appears to be irreversible. The mechanism of this effect is unclear. Whether this effect is due to the interaction of NBQ with the ribosome and subsequent damage to its organization or to the interaction of the compound with tRNA and/or mRNA remains to be determined.

To elucidate the effects of NBQ and fagaronine on the enzymes that polymerize nucleic acids, the activities of DNA and RNA polymerases were studied. The effect of NBQ on DNA and RNA polymerases was more pronounced than that elicited by fagaronine. Results obtained suggest that NBQ and fagaronine interfere with the role of DNA as a template in both transcription and replication. These results confirm those obtained by Sethi and coworkers [3, 4] which have shown that fagaronine inhibits DNA polymerase activity by interacting with the A:T template primer, and shows a moderate inhibition of RNA polymerase.

Attempts were made to explain the unusual response curves of NBQ and fagaronine that show strong inhibition of protein synthesis. These findings are not in agreement with those previously reported for other DNA-intercalating drugs like Adriamycin, Actinomycin D, CC-1065 and Mitonafide [5-nitro-2-(2-dimethylamino ethyl)-(benzode)-isoquinoline-1,3-dione], which preferentially inhibit nucleic acid synthesis whereas protein synthesis remains almost unaffected [11, 17, 21, 22]. Both compounds inhibited polypeptide synthesis in a cell-free system. Furthermore, the inhibition of [^{14}C]polyphenylalanine synthesis directed by polyuridine suggests that NBQ and fagaronine act at the elongation step

of protein synthesis. However, these data do not rule out the possibility that the compounds might act at other stages of polypeptide synthesis. These results provide strong evidence in favour of the hypothesis that fagaronine and NBQ act at two independent cellular sites. The interaction of NBQ and fagaronine with the protein-synthesizing machinery is not resolved. This may explain the inhibition of protein synthesis; however, the real significance of it in the cytoxicity and antitumour activity of both compounds remains to be evaluated. Further experiments to define this phenomenon are currently under way in our laboratory.

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