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Interaction of 2-Hydroxyamino-1,4-naphthoquinone with Nucleic Acid and Its Biological Actions

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As a part of the biological investigations on deoxyribonucleic acid (DNA)-interacting aminoquinones, the interaction of 2-hydroxyamino-1,4-naphthoquinone (HANQ) with nucleic acids and its biological actions were studied.

1. Spectroscopic determinations indicated that HANQ interacted with DNA without effect by Mg^{2+}. The interaction sites in DNA was estimated to be the purine bases from the difference spectra with nucleic acid derivatives.

2. HANQ inhibited thymidine incorporation into DNA in Ehrlich ascites carcinoma, rather than in *E. coli* cells, to a higher extent than mitomycin C. The precursor incorporation into RNA and proteins in the carcinoma was less inhibited; the inhibition resulted possibly from a secondary action of HANQ.

3. An antitumor effect of HANQ was observed on Ehrlich ascites carcinoma, ascites sarcoma-180, and L-1210 leukemia at doses less than half of the LD_{50} (mouse, *i.p.*, 318 mg/kg).

4. The biological actions of HANQ was discussed in analogy to 4-hydroxyaminoquinoline-1-oxide (4HAQO), a proximate carcinogen of 4-nitroquinoline-1-oxide (4NQO).

The significance of aminoquinone structure has been proposed by Rao, *et al.*[2] as a functional group of antitumor antibiotics, *e.g.*, actinomycins, mitomycins, porfiromycin, and streptonigrin. In agreement with the proposal, it has been reported from the authors' laboratory that several of simple aminoquinone compounds interact with deoxyribonucleic acid (DNA) and act as inhibitors of nucleic acid synthesis in Ehrlich ascites carcinoma.[3,4] Of these, 2-amino-1,4-naphthoquinone imine (ANQI) was a potent inhibitor of the DNA synthesis through the interaction with DNA.[3–5]

Since ANQI was oxidized in part in Ehrlich ascites carcinoma,[6] it is of interest to investigate the oxidation products of ANQI as the proximate substances of biological effects. The present paper deals with one of the oxidation products, 2-hydroxyamino-1,4-naphthoquinone (HANQ),[7] and presents studies on its interaction with nucleic acids and its biological actions. HANQ, a somewhat unstable hydroxyamino compound, also interacted with nucleic acids in a manner a little different from that of ANQI. It inhibited, as well as ANQI, DNA synthesis in Ehrlich ascites carcinoma *in vitro* and showed antitumor activities, which had not been observed with ANQI, on some species of experimental animal tumor.

1) Location: *2-2-1, Oshika, Shizuoka.*
2) K.V. Rao, K. Biemann, and R.B. Woodward, *J. Am. Chem. Soc.*, **85**, 2532 (1963).
3) S. Okada, *Chem. Pharm. Bull.* (Tokyo), **17**, 105 (1969).
4) S. Okada, *Chem. Pharm. Bull.* (Tokyo), **17**, 113 (1969).
5) S. Okada, *Chem. Pharm. Bull.* (Tokyo), **17**, 1057 (1969).
6) S. Okada and O. Tamemasa, *Chem. Pharm. Bull.* (Tokyo), **17**, 1432 (1969).
7) N. Mochizuki, S. Okada, and O. Tamemasa, *Yakugaku Zasshi*, **94**, 744 (1974).

Experimental

Materials——HANQ was synthesized in the authors' laboratory by the hydrogen peroxide oxidation of ANQI.[7] Calf thymus DNA (Type I, "highly polymerized"), yeast tRNA (Type III), poly A,[8] poly U,[8] and nucleoside-5′-monophosphates were purchased from Sigma Chemical Co. Apurinic and apyrimidinic calf thymus DNA were prepared according to the methods of Tamm, *et al.*[9] and of Takemura,[10] respectively. Heat-denatured calf thymus DNA was obtained by heating the native DNA at 100° for 15 min in 0.01M NaCl–0.01M tris[8]-HCl (pH 7.4) and then by cooling rapidly to 0°. Ribosomal RNA (rRNA) was isolated from Ehrlich ascites carcinoma by the hot phenol-SDS[8] method followed by a linear (5 to 40%) sucrose density gradient centrifugation in an RPS-25 rotor (Hitachi) at 25000 rpm for 16 hr.[11,12] Actinomycin S_3 was kindly furnished by Dr. J. Kawamata of Research Institute for Microbial Diseases, Osaka University. Thymidine-2-^{14}C, uridine-2-^{14}C, and L-phenylalanine-U-^{14}C were obtained from Daiichi Pure Chemicals Co., Ltd.

Absorption Spectra and Difference Spectra——These were measured by a Hitachi Recording Spectrophotometer, Model EPS-3T.

Alkaline Denaturation of DNA——Calf thymus DNA in 0.1×SSC[8] was titrated with 0.1N NaOH with recording the absorbance at 260 nm at each pH.

Ehrlich Ascites Carcinoma——The cells were harvested 7 days after the *i.p.* transplantation to male mice of strain dd/Y, weighed 20±2 g, and washed with Ca^{2+}-free modified Krebs-Ringer phosphate buffer (Ca^{2+}-free KRP),[13] pH 7.2, as described earlier.[14]

***E. coli*[8] Cells**——The cells of strain B grown in a glucose-salts synthetic medium[15] at 37° for 16 hr were collected and washed with Ca^{2+}-free KRP.

Incubation and Fractionation of Cells——The incorporation of a precursor into nucleic acids or proteins of Ehrlich ascites carcinoma or *E. coli* B cells was performed by incubation of the cells (0.1 g, wet weight) in 1.5 ml of Ca^{2+}-free KRP at 37° for 60 min with a radioactive precursor (final concentration, 1×10^{-4}M; specific radioactivity, 0.5 or 1 mCi/mmole) in the presence or absence of HANQ.

The fractionation of DNA and RNA was carried out according to the method of Schmidt, Thannhauser, and Schneider,[16] and that of proteins was performed as described earlier.[14]

Assay for Cell-Free Protein Synthesis——The protein synthesis in a cell-free system of Ehrlich ascites carcinoma was assayed as described by Littlefield, *et al.*[17] and Rendi, *et al.*[18] The incubation mixture contained in micromoles in a final volume of 1.5 ml: KCl, 300; $MgCl_2$, 15; tris-HCl buffer (pH 7.8), 150; ATP, 0.75; GTP, 0.12; β-mercaptoethanol, 120; creatine phosphate, 20; creatine phosphokinase, 70 μg; poly U, 10 μg; pancreatic deoxyribonuclease (Worthington Biochem. Corp.), 10 μg; L-phenylalanine-U-^{14}C (3 mCi/mmole), 0.15 (final concentration, 1×10^{-4}M); HANQ, as indicated; and postmitochondrial supernatant (15000×**g**, 10 min) of the tumor cells in the medium described by Littlefield, *et al.*[17] 0.5 ml. The incubation was carried out for 60 min at 37°.

Assay for Radioactivity and Incorporation of Precursor——The radioactivity was assayed in a windowless 2π-gas-flow counter as described earlier.[14] The amount of a precursor incorporated was calculated also as reported in the previous paper.[14]

Acute Toxicity in Mice——On the basis of the mortality of mice 24 hr after *i.p.* injection of HANQ, suspended in 0.5 ml of physiological saline containing 1% carboxymethylcellulose, the value of LD_{50} was determined according to the method of van der Waerden.[19]

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8) Abbreviations: poly A—polyadenylic acid, poly U—polyuridylic acid, tris—tris-(hydroxymethyl)-aminomethane, SDS—Na-dodecylsulfate, 0.1×SSC—0.015M NaCl–0.0015M Na-citrate buffer, pH 7.4, *E. coli*—*Escherichia coli*, 5′-AMP—adenosine-5′-monophosphate, 5′-GMP—guanosine-5′-monophosphate, 5′-CMP—cytidine-5′-monophosphate, 5′-UMP—uridine-5′-monophosphate, 5′-dTMP—deoxythymidine-5′-monophosphate.

9) C. Tamm, M.E. Hodes, and E. Chargaff, *J. Biol. Chem.*, **195**, 49 (1952).
10) S. Takemura, *Bull. Chem. Soc. Japan*, **32**, 920 (1959).
11) C.M. Mauritzen, Y.C. Choi, and H. Busch, "Methods in Cancer Research," Vol. 6, ed. by H. Busch, Academic Press, New York, 1971, pp. 253—282.
12) S. Okada and H. Busch, *Cancer Res.*, **32**, 1737 (1972).
13) M. Rabinovitz, M.E. Olson, and D.M. Greenberg, *J. Biol. Chem.*, **210**, 837 (1954).
14) O. Tamemasa, S. Okada, and Y. Wakita, *Chem. Pharm. Bull.* (Tokyo), **13**, 1193 (1965).
15) Composition of glucose-salts synthetic medium: glucose, 3.0 g/liter; Na-glutamate, 3.0 g/liter; NaCl, 5.0 g/liter; $MgSO_4\cdot7H_2O$, 0.1 g/liter; $(NH_4)_2HPO_4$, 2.5 g/liter; KH_2PO_4, 1.5 g/liter; pH 7.2.
16) G. Schmidt and S.J. Thannhauser, *J. Biol. Chem.*, **161**, 83 (1945); W.C. Schneider, *ibid.*, **161**, 293 (1945); W.C. Schneider, *ibid.*, **164**, 747 (1946); S. Mizuno, *Kagaku To Seibutsu*, **3**, 148 (1965).
17) J.W. Littlefield and E.B. Keller, *J. Biol. Chem.*, **224**, 13 (1957).
18) R. Rendi and S. Ochoa, *J. Biol. Chem.*, **237**, 3707 (1962).
19) B.L. van der Waerden, *Arch. Exptl. Pathol. Pharmakol.*, **195**, 389 (1940).

Antitumor Activity——Two species of mouse ascites carcinoma and one of mouse leukemia were tested; Ehrlich ascites carcinoma in dd/Y mouse, ascites sarcoma-180 in dd/N mouse, and L-1210 leukemia in BDF_1 mouse.

Ehrlich ascites carcinoma (approximately 2×10^6 cells) were transplanted *i.p.* into each mouse. HANQ suspended in physiological saline containing 1% carboxymethylcellulose was then injected *i.p.* once a day for 7 days starting 24 hr after the transplantation. The antitumor activity was determined from the survival days and the change of body weight of each mouse.

The tests on sarcoma-180 and L-1210 were cooperated by Drs. K. Kuretani and A. Hoshi of National Cancer Center Research Institute. The numbers of the cells transplanted into each mouse were 1×10^7 (sarcoma-180) and 1×10^5 (L-1210). The transplantation of the cells and the administration of HANQ were conducted in the same manner as the case of Ehrlich ascites carcinoma except that the number of HANQ injection was once a day for 5 days. The activity was evaluated by the total packed cell volume (TPCV) ratio (T/C%)[20] on the 7th day in sarcoma-180 and by the increase in life span (ILS) in L-1210.[21]

Result

Interaction with DNA

As shown in Fig. 1, calf thymus DNA had a hyperchromic effect on HANQ throughout the visible region. This indicates the interaction of HANQ with DNA.

The interaction could also be demonstrated by measurement of helix-coil transition of DNA. Since HANQ was rather unstable in a neutral solution at 37°, *i.e.*, the absorbance at λ_{max} (560 nm) decreased to *ca.* 50% in 1.5 hr, the method of alkaline denaturation of DNA, instead of heat denaturation, was applied to see the effect of HANQ. As shown in Fig. 2, the hyperchromicity of calf thymus DNA in the alkaline region was remarkably reduced by the presence of HANQ.

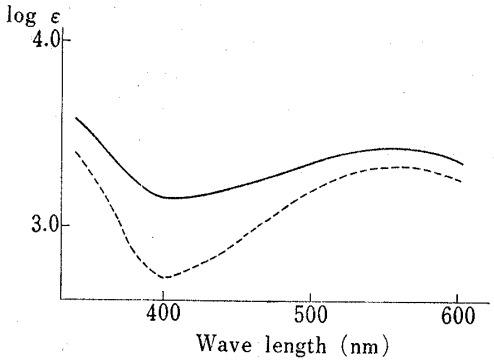

Fig. 1. Spectral Change of HANQ by Calf Thymus DNA

——: HANQ (1×10^{-4}M in 0.01M NaCl–0.01M Tris-HCl, pH 7.4) with DNA (1×10^{-3}M as nucleotide)
----: without DNA

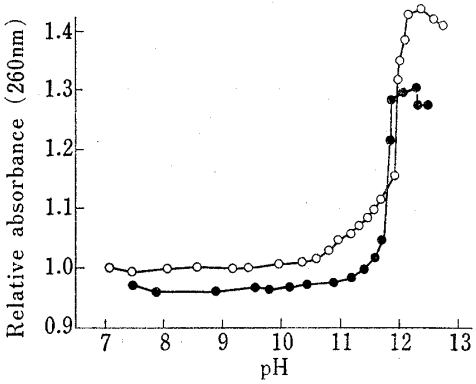

Fig. 2. Effect of HANQ on Alkaline Denaturation of DNA

In the presence (—●—) or absence (—○—) of 1×10^{-4}M HANQ, calf thymus DNA (1×10^{-4}M, as nucleotide) in 0.1×SSC was Titrated with 0.1N NaOH. Hyperchromicity of DNA with and without HANQ is 33.7% and 43.7%, respectively.

Interaction Sites of DNA

Fig. 3 shows the difference spectra of HANQ and nucleic acids against HANQ. The visible spectrum of HANQ was shifted by native and apyrimidinic calf thymus DNA as well as Ehrlich ascites carcinoma rRNA. The shift by native calf thymus DNA, the most prominent one, was not affected by the presence of Mg^{2+}. This suggests that the mode of interaction of HANQ with DNA may differ from those of the other aminoquinones, *i.e.*, 2-amino-3H-phenoxazin-3-one, 2-amino-1,4-benzoquinone imine, and ANQI, whose bindings to DNA

20) E.N. Sassenrath, *Ann. N.Y. Acad. Sci.*, **76**, 601 (1958).
21) A. Hoshi, F. Kanzawa, and K. Kuretani, *Cancer Chemother. Rep.*, Part 1, **55**, 229 (1971).

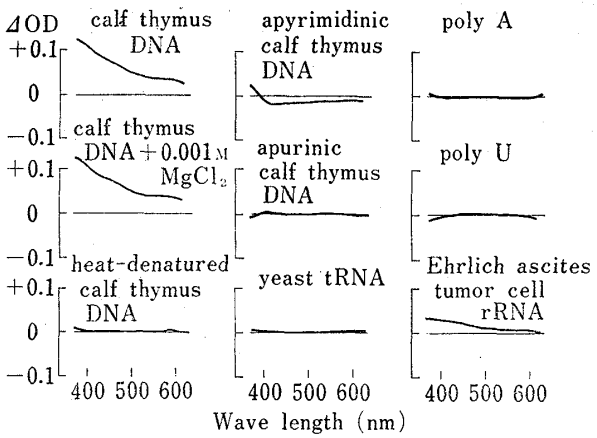

Fig. 3. Difference Spectra of HANQ and Nucleic Acid Derivatives against HANQ

Concentrations of HANQ and nucleic acid (as nucleotide) are 1×10^{-4}M and 1×10^{-3}M, respectively, in 0.01M NaCl–0.01M Tris-HCl, pH 7.4.

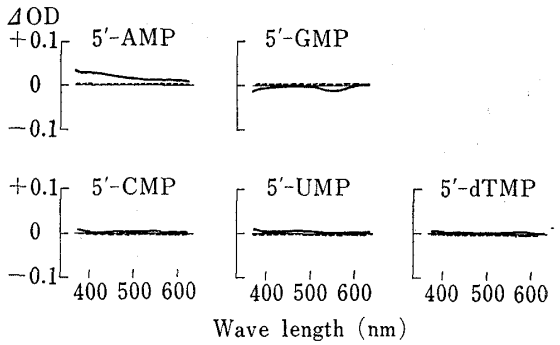

Fig. 4. Difference Spectra of HANQ and Nucleoside-5′-monophosphates against HANQ

Concentrations of HANQ; 1×10^{-4}M and nucleoside-5′-monophosphates; 1×10^{-2}M (——) and 1×10^{-3}M (----) in 0.01M NaCl–0.01M Tris-HCl, pH 7.4.

were reduced by the presence of Mg^{2+} as reported previously.[3] On the other hand, no appreciable change was observed in the spectrum of HANQ by the addition of the heat-denatured DNA, apurinic calf thymus DNA, poly A, and poly U.

Interaction with Nucleotide

A possibility that the interaction sites of DNA were the purine bases, which was postulated by the spectral change of HANQ by the apyrimidinic DNA, not by the apurinic DNA (Fig 3), became more likely by an experiment on nucleoside-5′-monophosphates. As shown in Fig. 4, the visible spectrum of HANQ was shifted appreciably by purine nucleoside-5′-monophosphates, *i.e.*, 5′-AMP and 5′-GMP, at a concentration 10 times higher than that of nucleic acid, whereas it was not changed by pyrimidine nucleoside-5′-monophosphates at any concentration tested.

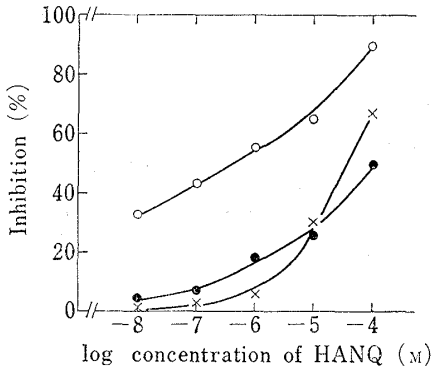

Fig. 5. Inhibition of Precursor Incorporation by HANQ into DNA, RNA, and Proteins of Ehrlich Ascites Carcinoma *in Vitro*

The cells (0.1 g, wet weight) were incubated at 37° for 60 min with a radioactive precursor (1×10^{-4}M) in the presence of HANQ in 1.5 ml of Ca^{2+}-free KRP.
—○—: thymidine-2-^{14}C into DNA
—●—: uridine-2-^{14}C into RNA
—×—: L-phenylalanine-U-^{14}C into proteins

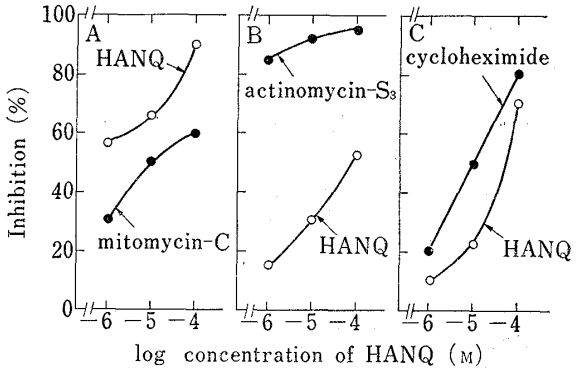

Fig. 6. Inhibition of Precursor Incorporation by HANQ or Antibiotics into DNA, RNA, and Proteins of Ehrlich Ascites Carcinoma *in Vitro*

The cells (0.1 g, wet weight) were incubated at 37° for 60 min with a radioactive precursor (1×10^{-4}M) in the presence of HANQ or an antibiotic in 1.5 ml of Ca^{2+}-free KRP.
A: thymidine-2-^{14}C into DNA
B: uridine-2-^{14}C into RNA
C: L-phenylalanine-U-^{14}C into proteins

Effect on DNA, RNA, and Protein Synthesis in Ehrlich Ascites Carcinoma *in Vitro*

As in the cases of the other aminoquinones[3,5] and DNA-interacting antibiotics, HANQ could be expected to affect the synthesis of nucleic acids or proteins in carcinoma. As shown in Fig. 5, the incorporation of thymidine into DNA of Ehrlich ascites carcinoma was highly inhibited by HANQ. As much as 90% of the thymidine incorporation was inhibited by 1×10^{-4}M HANQ and approximately 30% even at 1×10^{-8}M. The extent of the inhibition was evidently higher than that by mitomycin C (Fig. 6). On the other hand, the incorporation of both uridine and L-phenylalanine into RNA and proteins, respectively, was inhibited to much lesser extents (Fig. 5). The percentages of these inhibitions were lower than those by actinomycin S_3 and cycloheximide as shown in Fig. 6.

Fig. 7 presents the percent inhibition as a function of incubation time. By 5 min incubation with 1×10^{-4}M HANQ the inhibition of thymidine incorporation into DNA already reached to *ca.* 60% without a marked increase by further incubation. The inhibition of uridine and L-phenylalanine incorporation, on the contrary, was successively increased up to 60 min. The delay in the inhibition suggests that the incorporation of uridine and L-phenylalanine into RNA and proteins, respectively, was inhibited probably by one of the secondary actions of HANQ in the intact cells. With respect to protein synthesis, this suggestion was realized by an experiment of poly U-directed cell-free protein synthesis; HANQ at concentrations of 1×10^{-6}—1×10^{-4}M had no effect on the cell-free incorporation of L-phenylalanine into protein.

Effect on DNA, RNA, and Protein Synthesis in *E. coli*

As shown in Fig. 8, the synthesis in *E. coli* B cells was also inhibited by HANQ. The inhibition rate of the DNA synthesis, however, was evidently lower than that in the case of Ehrlich ascites carcinoma (Fig. 5).

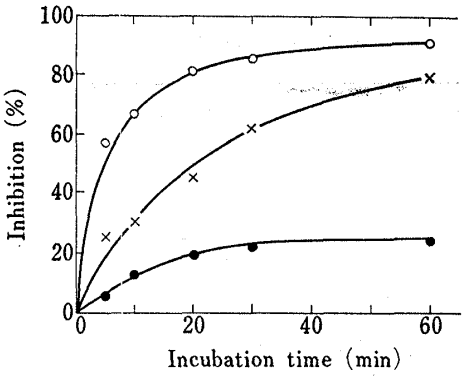

Fig. 7. Time Course of HANQ Inhibition of Precursor Incorporation into DNA, RNA, and Proteins of Ehrlich Ascites Carcinoma *in Vitro*

The cells (0.1 g, wet weight) were incubated at 37° with a radioactive precursor (1×10^{-4}M) in the presence of 1×10^{-4}M HANQ in 1.5 ml Ca^{2+}-free KRP. Each of percent inhibition to control is plotted as a function of incubation time.
—○—: thymidine-2-^{14}C into DNA
—●—: uridine-2-^{14}C into RNA
—×—: L-phenylalanine-U-^{14}C into proteins

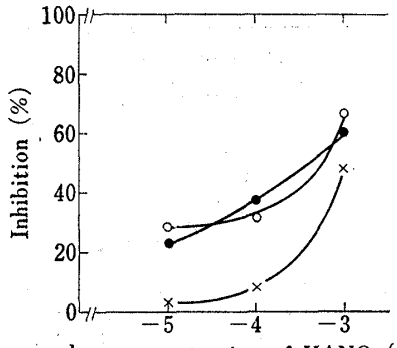

Fig. 8. Inhibition of Precursor Incorporation by HANQ into DNA, RNA, and Proteins of *E. coli*

The cells (0.1 g, wet weight) were incubated at 37° for 60 min with a radioactive precursor (1×10^{-4}M) in the presence of HANQ in 1.5 ml of Ca^{2+}-free KRP.
—○—: thymidine-2-^{14}C into DNA
—●—: uridine-2-^{14}C into RNA
—×—: L-phenylalanine-U-^{14}C into proteins

Antitumor Activity

As shown in Table I, the value of LD_{50} obtained in mice 24 hr after *i.p.* injection of HANQ was 318 (264—383) mg/kg. Since the value indicated that the acute toxicity of HANQ was much lower than that of ANQI (LD_{50}=5.45 mg/kg, mouse, *i.p.*),[6] it seemed worthwhile to test the antitumor activity.

Table I. Acute Toxicity of HANQ in Mice

Dose of HANQ injected *i. p.* (mg/kg)	Mortality[a] (Survived / Administered)
100	6/6
200	5/6
400	2/6
800	0/6
LD_{50}=318 (264—383) mg/kg	

a) 24 hr after the injection

Table II. Effect of HANQ on Ehrlich Ascites Carcinoma

Dose[a] (mg/kg/day)	No. of 60-day survivors / Total No. of test animals	Average survival time of dead mice
Control	0/8	14.2 days
80	2/8	22.3
100	2/8	22.8
120	3/8	27.2
140	2/8	30.8
160	2/8	43.8

a) HANQ suspended in physiological saline containing 1% carboxymethylcellulose was *i.p.* injected into dd/Y mice once a day for 7 days starting 24 hr after transplantation of the carcinoma (*ca.* 2×10^6 cells/mouse).

Table III. Effect of HANQ on Mouse Ascites Sarcoma-180

Dose[a] (mg/kg/day)	Tumor growth[b] (T/C %)	Activity[c]
30	75	—
100	14	++
300	0	toxic

a) HANQ suspended in physiological saline containing 0.5% carboxymethylcellulose was *i.p.* injected into dd/N mice (6/ group) once a day for 5 days starting 24 hr after transplantation of the carcinoma (*ca.* 1×10^7/mouse).
b) Tumor growth (T/C %) was expressed as percent ratio of total packed cell volume (TPCV) on the 7th day to control animals.[20]
c) Antitumor activity was graded as —100—66, +65—41, ++ 40—11, +++ 10—0 of T/C%.

Table IV. Effect of HANQ on Mouse L-1210 Leukemia

Dose[a] (mg/kg/day)	Increase in life-span[b] (ILS %)	Activity[c]
100	21	++
200	9	— (toxic)

a) HANQ suspended in physiological saline containing 0.5% carboxymethylcellulose was *i.p.* injected into BDF_1 mice (6/group) once a day for 5 days starting 24 hr after transplantation of the leukemic cells (*ca.* 1×10^5/mouse).
b) ILS % was expressed as percent increase in life-span to control animals.[21]
c) Antitumor activity was graded as —0—9, +10—19, ++20—29, +++30 or more in ILS %.

Table II shows that the daily *i.p.* administration of HANQ at less than half the dose of LD_{50} prolonged the survival days of mice transplanted with Ehrlich ascites carcinoma. At any dose tested, the tumor growth in 2 to 3 mice out of 8 in a group was completely inhibited and the survival periods of those mice were at least a few months. The daily administration of 100 mg/kg of HANQ for 5 days also caused a suppression in the growth of ascites sarcoma-180 in dd/N mice (Table III) or an elongation of the life-span of BDF_1 mice transplanted with L-1210 leukemic cells (Table IV). However, a higher dose of HANQ, 200 (L-1210) or 300 mg/kg/day (sarcoma-180), was toxic to both species of the tumor-bearing mice (Table III, IV).

Discussion

The present study demonstrates that HANQ interacts with DNA and potentially inhibits the incorporation of thymidine into DNA of Ehrlich ascites carcinoma *in vitro*. The modes of the interaction and the inhibition appeared to be similar to those by ANQI as far as observed by the methods employed in this study; both HANQ and ANQI were assumed to interact with purine base moieties of DNA (Fig. 3, 4)[4] and both inhibited DNA synthesis to a similar extent rather than RNA and protein synthesis (Fig. 5—7).[3,5]

However, some differences in the interaction with DNA were concurrently observed between HANQ and ANQI. The binding of DNA caused a hyperchromicity in the spectrum of HANQ throughout the visible region (Fig. 1, 3), whereas it produced a red-shift in that of ANQI.[4] The interaction of HANQ with DNA, on the other hand, was not affected by the presence of Mg^{2+} (Fig. 3), while that of ANQI was reduced by Mg^{2+}.[4] These results suggest that the mode of the interaction of HANQ may not be identical to that of ANQI, though it is rather difficult to find out the more detailed manner of the interaction of HANQ by means of spectrometry mainly because of its instability in a neutral solution.

Although the mechanisms of the inhibition of thymidine incorporation into DNA of Ehrlich ascites carcinoma remain obscure, a possibility exists that HANQ taken up by the cells was transferred to the chromatin, interacted with the DNA and inhibited the DNA synthesis. This possibility is further confirmed by preliminary experiments, which will be reported later, on the cell-free DNA polymerase reaction and on the strand-scission effect of HANQ on cellular DNA.

A difference remarkable between HANQ and ANQI is that HANQ (100—200 mg/kg/day, *i.p.*) showed antitumor effect on some of experimental tumors of mice (Ehrlich ascites carcinoma, ascites sarcoma-180, and L-1210; Table II—IV) in contrast to the ineffectiveness of ANQI under the doses below its LD_{50} (mouse, *i.p.*, 5.45 mg/kg).[6] This is likely due to the low acute toxicity of HANQ (mouse, *i.p.*, 318 mg/kg; Table I), which is one of the requirements for chemotherapeutic agents. Although the antitumor activity and the chemotherapeutic coefficient of HANQ were not sufficient, it seems worthwhile to investigate its derivatives for the development of cancer chemotherapy.

When HANQ is compared with 4-hydroxyaminoquinoline-1-oxide (4HAQO) which has been estimated as a proximate carcinogen of 4-nitroquinoline-1-oxide (4NQO),[22] several similarities can be found. Both hydroxyamino compounds are planar in structure with almost the same molecular size and rather unstable, interact with DNA, if not by themselves but by biologically modified products, and possibly cause strand-scission of DNA.[22] Furthermore, antitumor activity has been reported on some of 4HAQO derivatives.[23] From

22) "Chemistry and Biological Actions of 4-Nitroquinoline-1-oxide," ed. by H. Endo, T. Ono, and T. Sugimura, Springer, Berlin, 1971.
23) Y. Kawazoe, M. Araki, Y. Nagata, and S. Miura, "Proc. Japan. Cancer Assoc., 32nd Ann. Meeting, Oct. 1973," Tokyo, p. 201.

these considerations, HANQ could be a compound of both biological and cancer-chemotherapeutic interests.

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