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Polyclonal Antibodies to DNA Modified with 4-Nitroquinoline 1-Oxide: Application for the Detection of 4-Nitroquinoline 1-Oxide-DNA Adducts *in vivo*

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Antibodies against 4-nitroquinoline 1-oxide (4NQO) adducts were elicited in rabbits immunized with 4NQO-modified DNA complexed with methylated bovine serum albumin. In enzyme-linked immunosorbent assay (ELISA), the antibodies could recognize either denatured or native 4NQO-modified DNA, but not unmodified DNA, DNA modified with other carcinogens or free 4NQO derivative. Modification levels as low as 5 μmol of adduct per one mole DNA nucleotide (5 adducts/10^6 nucleotides) can be easily detected by the competitive ELISA. Indirect immunofluorescence staining by anti 4NQO-DNA antibody indicated that the antibodies bound specifically to the nuclei of normal human skin fibroblast cells treated with 4NQO. The intensity of fluorescence was proportional to the dose of 4NQO used to treat the cells, and the fluorescence-positive cells could be detected after treatment with 0.25μM 4NQO (which resulted in the formation of 10^4 adducts per cell). Applying the competitive ELISA to the quantitation of DNA-adducts in rats treated with 4NQO, it was confirmed that the sensitivity of immunochemical assays was equivalent to that of isotopic assays. These methods should be helpful in studies on the formation of adducts and their removal in cells and tissues.

Key words: Antibodies — 4NQO-DNA — Immunoassay — Immunostaining

It is well-known that ultimate carcinogens bind covalently to DNA and it is generally thought that this chemical modification is the critical step in the initiation of the tumorigenic process.[1] In general, several kinds of adducts are formed in DNA by treatment with carcinogens, but it is not yet known how these adducts can lead to mutational and transformational events. 4-Nitroquinoline 1-oxide (4NQO),[*6] as well as acetylaminofluorene[2, 3] and benzo[*a*]pyrene[4] have received great attention among the known carcinogens. The advantages of using 4NQO as a chemical are that its metabolic activation systems commonly exist in prokaryotic and eukaryotic cells.[5] It has been shown that 4NQO binds covalently to guanine and adenine residues in cellular DNA, producing three guanine adducts, namely QGI, QGII and QGIII, and one adenine adduct, QA. Among these adducts, QGI has been identified as N-(guanine-8-yl)-4AQO (Guo-C8-AQO), QGIII as 3-(guanine-N^2-yl)-4AQO (Guo-N^2-AQO), and QA as 3-(adenine-N^6-yl)-4AQO.[6-8]

These 4NQO-purine adducts are excised from the DNA of wild-type *E. coli* as well as of normal human cells, but not from the DNA in *E. coli* excision-repair deficient strain (uvrA$^-$) or xeroderma pigmentosum cells, which can not excise pyrimidine dimers.[9, 10] From these UV-mimetic characteristics of 4NQO, it seems likely that 4NQO damage to DNA is subjected to nucleotide excision, as are pyrimidine dimers. Consequently, 4NQO-purine adducts are considered to be the main cause of the biological action of 4NQO, such

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[*6] Abbreviations used are: 4NQO, 4-nitroquinoline 1-oxide; 4HAQO, 4-hydroxyaminoquinoline 1-oxide; 4AQO, 4-aminoquinoline 1-oxide; 4NQO-DNA, 4NQO-modified DNA; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; NGS, normal goat serum; PBS, phosphate-buffered saline solution (pH 7.3).

as killing, mutation and transformation.[6, 9, 10] Recent studies have shown that the level and persistence of specific adducts in a 'target' cell correlate well with oncogenicity.[11-13]

The availability of methods for direct measurement of specific DNA adducts would greatly facilitate studies on the biological significance of these lesions. Various methods, e.g., highly sensitive chemical analyses using HPLC, or isotopic analyses with radioactive chemicals, have been developed to measure DNA adduct formation and removal after treatment of animals or cultured cells with carcinogens. In recent years, the immunochemical approach to detect DNA damage has been developed because of its sensitivity and specificity.[14-18] The use of polyclonal rabbit antibodies as well as monoclonal antibodies for highly sensitive and specific immunoassay has led to the detection of femtomole levels of carcinogen in microgram amounts of DNA.

Here, we report on the development and characterization of rabbit polyclonal antibodies to 4NQO-modified DNA (4NQO-DNA), and the optimal conditions for the sensitive assay of DNA adducts were established. By using immunohistochemical techniques, we have examined the formation and removal of these lesions from DNA in cultured human cells. The competitive enzyme-linked immunosorbent assay (ELISA) was applied to the study of DNA adduct formation in rats treated with 4NQO.

Materials and Methods

Chemicals [^{3}H]4NQO and [^{3}H]4HAQO were kindly donated by Dr. Y. Kawazoe of Nagoya City University. Normal human skin fibroblast cells, NHSF(6), were kindly provided by Dr. Y. Fujiwara, Kobe University. Seryl-AMP was prepared according to Berg.[19] 4-Nitroquinoline 1-oxide, 4-hydroxyaminoquinoline 1-oxide (4HAQO), 4-aminoquinoline 1-oxide (4AQO) and single-stranded DNA cellulose were obtained from Wako Pure Chemicals Ind. Ltd., Osaka. Goat anti-rabbit IgG-peroxidase and IgG-fluorescein isothiocyanate (FITC) were obtained from Tago Inc., Burlingame, Ca. Complete and incomplete Freund's adjuvant were obtained from Difco Lab., Detroit, Mich.

Preparation of Modified DNA Commercial native calf thymus DNA was modified *in vitro* with 4HAQO by the following procedure[20]: DNA (0.5 mg/ml) in 20m*M* Tris-HCl (pH 8.0) was incubated with 0.5m*M* [^{3}H]4HAQO (0.2 mCi/mol), 2m*M* seryl-AMP and 1m*M* DTT for 30 min at 37°. The nonreacted 4HAQO derivatives were removed by extraction with Tris-buffer (pH 8.0) saturated redistilled phenol and diethyl ether, each extraction being performed twice. The modified DNA was then dialyzed against 0.15*M* NaCl in 20m*M* Na-phosphate buffer (pH 7.0). A series of standard DNA preparations modified with 4NQO (from 0.024 adducts to 6 adducts per 10^3 nucleotides) were prepared with [^{3}H]4HAQO in the concentration range from 0.5μM to 0.5m*M*. The amount of [^{3}H]4NQO adducts in DNA was quantified by using a liquid scintillation system. DNA was determined by measuring the absorbance at 260 nm ($\varepsilon = 6500$). The DNA hydrolyzates were quantitatively analyzed for specific adducts with paper chromatography, as described previously.[6]

Preparation of Rabbit Immunoglobulin against 4NQO-modified DNA The 4NQO-DNA (250 μg) was electrostatically complexed to an equal amount of methylated bovine serum albumin (mBSA) in phosphate-buffered saline (PBS) emulsified in an equal volume of complete Freund's adjuvant and injected into the footpads of a female Japanese rabbit weighing 2.5 kg. Each rabbit was subsequently immunized subcutaneously at 14-day intervals for two months with 250 μg of 4NQO-DNA-mBSA complex with incomplete Freund's adjuvant.

After 3 months, the same antigen mixture without adjuvant was injected subcutaneously. Blood samples were taken at one week after the last injection from the ear vein of 2 immunized rabbits and the sera were tested for antibody activity by ELISA (see below). Antisera from the two rabbits showed about the same specificity. Sera from No. 2 rabbit, which showed higher activity than No. 1 rabbit, were precipitated by the addition of one-half volume of saturated ammonium sulfate (pH 7.2, at 20°). The resulting precipitates were collected and dissolved in PBS and dialyzed against 10m*M* sodium phosphate buffer (pH 7.0). The dialyzate was applied to a column of DEAE-Sepharose CL-6B (Pharmacia) and eluted with 50 m*M* phosphate buffer. IgG fractions were dialyzed against 0.1*M* NaCl-20m*M* Tris-HCl (pH 7.2)-1m*M* EDTA. Then, the dialyzate was passed through a single-stranded DNA cellulose column, and the noncharged fraction was collected and dialyzed against 0.1*M* NaCl in borate buffer (pH 8.0). The final, dialyzed fraction was used as the antibody fraction.

Enzyme-linked Immunosorbent Assay (ELISA)[21] Polystyrene U-bottomed microtiter wells (Immunoplate Type II, Nunc, Roskild, Denmark) were routinely coated with 50 ng of 4NQO-DNA,

containing 0.6% modified bases, in 50 μl of PBS, and dried overnight at room temperature. After blocking nonspecific binding with 0.4 ml of 0.02% gelatin solution for one hour at 37°, the plates were washed 5 times with PBS containing 0.05% Tween 20 (wash medium). Then, 50 μl of IgG fraction diluted in PBS containing 1% FCS was added to each well. The plate was incubated at 37° for one hour, and washed 5 times with wash medium. Each well was incubated with 50 μl of peroxidase-conjugated goat anti-rabbit IgG (diluted in PBS containing 1% FCS) for one hour at 37°. After 5 washes with wash medium, the wells were finally incubated for 30 min at 37° with 50 μl of substrate solution for the enzyme (4 mg of *o*-phenylenediamine and 2 μl of H_2O_2 in 10 ml of reaction buffer, pH 5.2); 100 μl of 2.5*M* H_2SO_4 was added to stop the reaction. The absorbance at 492 nm was recorded with a microplate measurement system (Shimadzu CS-930 dual-wavelength TLC scanner). Blank readings were taken from those wells in which PBS containing 1% FCS had been added instead of antisera.

In the competitive assay, the competitive mixtures (100 μl) containing the antiserum and serial dilutions of the inhibitors were transferred to the assay wells and incubated to allow for binding of the antibody remaining free after the pre-incubation. Calibration curves were constructed by pre-incubating the antibody with various amounts of 4NQO-DNA.

Treatment of Normal Human Skin Fibroblasts with 4NQO Normal human skin fibroblast cells were grown as monolayer cultures on Lab-Tek 2 chamber-slides (Lab-Tek Products, Naperville, Ill.) in Dulbecco's modified minimal essential medium (D-MEM) supplemented with 10% fetal calf serum (GIBCO) and 10m*M* HEPES buffer (pH 7.2). Prior to 4NQO treatment, the cells were washed twice with PBS. The stock solution of 4NQO in 0.01*N* HCl was diluted to appropriate concentrations in Hanks' solution. Each well was provided with a 1.0 ml aliquot. Control cells were treated with Hanks' solution without carcinogen. After exposure for one hour at 37° in an atmosphere of 5% CO_2-95% air, the cells on the chamber-slides were quickly washed with PBS. They were fixed for 15 min at 4° in 4% paraformaldehyde and treated with graded ethanol up to 90% in PBS. Then, they were successively washed with PBS containing 0.5% Tween 20, PBS and 2×SSC. In the processing of the cells for immunostaining, the cells were treated with a mixture of RNase A (100 μg/ml) and RNase T_1 (50 units/ml) in 2×SSC at 37° for 45 min. After the incubation, the slides were washed three times with PBS at 5 min per washing. To remove the chromatin protein *in situ*, the slides were incubated in 2.5*N* HCl for 45 min and neutralized with sodium borate-buffered solution. Then, they were washed with PBS (3×5 min) and used for immunostaining.

Slides were covered with 100 μl of 5% normal goat serum (NGS). After removal of the NGS, the slides were incubated with 100 μl of the rabbit anti-4NQO-DNA IgG solution (2–20 μg in PBS containing 5% NGS) for 60 min at 37° and washed with PBS (3×5 min). They were further incubated for 60 min at 37° with FITC-conjugated goat-anti rabbit IgG, diluted 1:50 in PBS containing 5% NGS. Unbound antibodies were removed by washing with PBS (3×5 min). The slides were mounted in glycerol/PBS (9:1), containing *p*-phenylendiamine, cover-slipped and examined by means of phase contrast and fluorescence microscopy using a Nikon fluorescence microscope (Fluophot UFX).

Treatment of Rats with 4NQO; Isolation of DNA [^{3}H]4HAQO (28 mCi/mmol) was intravenously administered to female rats (strain Donryu, average weight 180–200 g) at dosages of 20 mg per kg body weight. After one hour, the DNA was isolated from the liver, pancreas, kidney, lung and uterus by means of sodium dodecyl sulfate and phenol extraction and RNase treatment.[6] The amount of 4NQO adducts in DNA was measured as described above. For the competitive ELISA, the DNA samples were heated for 3 min at 90° and rapidly cooled on ice.

Results

Antibody-binding to Immobilized 4NQO-DNA The modification level of DNA by 4NQO, used as an immunogen, was 0.6% (6 4NQO adducts in 10^3 nucleotides) and chromatographic analysis of the acid hydrolyzate of DNA confirmed that three guanine (QGI, QGII and QGIII) and one adenine (QA) adducts were produced in the ratios of 6.6, 57.2, 16.4 and 19.7%, respectively.

The IgG preparations obtained after partial purification by precipitation with ammonium sulfate, followed by DEAE-Sepharose and DNA-cellulose chromatography, were used in the experiments described below. A single-stranded DNA-cellulose column chromatography step was used to remove the fraction of antibodies that reacted with unmodified single-stranded DNA.

Direct ELISA was utilized to reveal the specificity of the antibodies.[21] Microtiter plates were coated with 50 ng of 4NQO-modified or unmodified, denatured or native DNA. Specific binding could be detected at 25

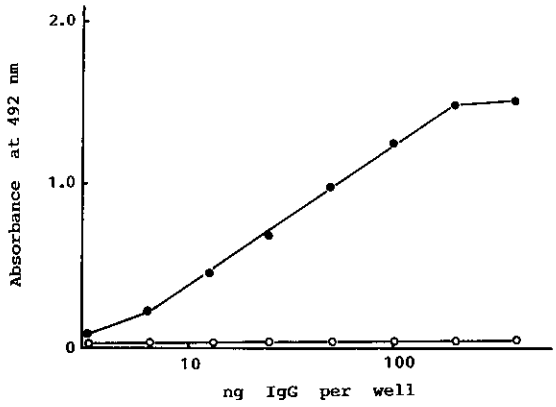

Fig. 1. Specificity of anti-4NQO-DNA antibodies. The wells were coated with 4NQO-DNA (0.6% modified bases) and increasing amounts of anti 4NQO-DNA IgG (●) and IgG from nonimmunized rabbits (○) were used.

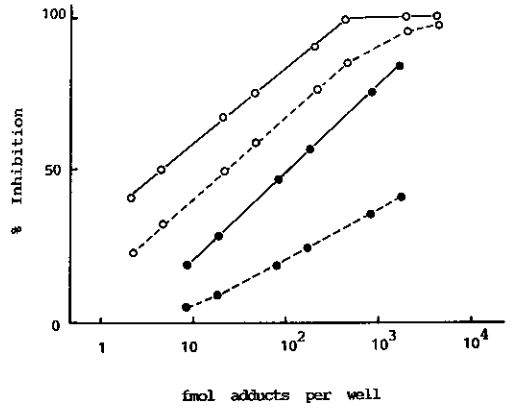

Fig. 3. Percentage inhibition of antibody binding in the competitive ELISA by native (----) and heat-denatured (——) 4NQO-DNA's as inhibitors. The modification levels of the 4NQO-DNA were 0.0024% (●) and 0.06% (○). Wells were coated with 50 ng of native 4NQO-DNA containing 0.6% modified bases, and 25 ng of IgG was used.

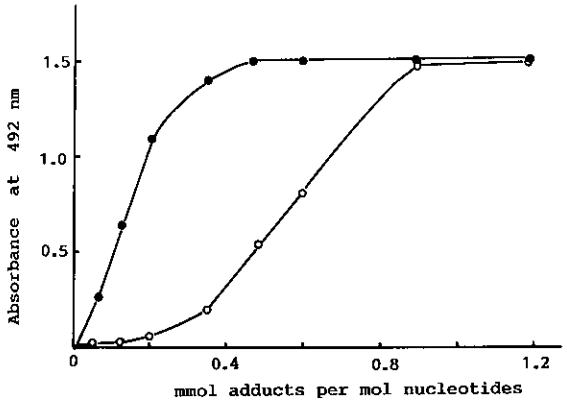

Fig. 2. Antibody binding to various amounts of immobilized antigen. Each well in the microtiter plate was coated with 30 ng of DNA containing various amounts of 4NQO-modified bases as indicated on the abscissa, and 25 ng of IgG fraction was applied (see "Materials and Methods"). Antibody binding was measured by ELISA and expressed as absorbance at 492 nm. Native (○) and heat-denatured (●) 4NQO-DNA.

ng of IgG fraction. Nonspecific binding of IgG to unmodified DNA was not observed even when using 1 μg of antibody. IgG purified from nonimmune sera did not bind to 4NQO-DNA (Fig. 1). To determine the optimal conditions for ELISA, the antibodies were tested against immobilized DNA containing various amounts of adducts. As shown in Fig. 2, antibody-binding increases with increasing modification level of DNA. This antibody gives higher reactivity with modified, denatured DNA (90° for 3 min) compared to the native form, and can detect 25 adducts per 10^5 nucleotides in native DNA and 5 adducts per 10^5 nucleotides in denatured DNA at 30 ng per well. When the modification level was higher than 0.1%, native and denatured DNA gave the same sensitivity in the assay.

When the microtiter wells were coated with 4NQO-DNA at concentrations between 20 and 50 ng per well, the optimal assay condition for ELISA was obtained. Below 20 ng of DNA, the binding efficiency of antibody decreased with decreasing DNA concentration. With greater amounts of modified DNA, that is over 100 ng per well, a gradual decrease was seen in the absorbance at 492 nm. This is typical of the decrease in detection seen at high antigen levels in ELISA.[22, 23]

Specificity of the Antibodies We used competitive ELISA for the characterization of the antibodies. As shown in Fig. 3, these antibodies show higher reactivity to the modified denatured DNA as compared to native DNA with a similar level of modification, but no

Table I. Competitive Inhibition of Rabbit Antibodies against 4NQO-DNA[a]

Compound		Amount required for 50% inhibition (fmol)
dGuo-8-yl-4AQO		250
dGMP-8-yl-4AQO		3,500
dGuo		$>1\times10^5$[b]
dGMP		$>1\times10^5$
4NQO		$>1\times10^5$
4HAQO		$>1\times10^5$
4AQO		$>1\times10^5$
4NQO-modified		
Heat denatured DNA[c]	(0.6)[d]	5[e]
Alkali-denatured[f]	(0.6)	1.65
Native DNA	(0.6)	20
Poly(dG-dC)·poly(dG-dC)	(4.2)	2,120
RNA	(1.2)	3,800
Polyguanylic acid	(2.4)	2,400
Polyadenylic acid	(0.9)	10,500
Nuclease P_1-digested DNA	(0.6)	120
Heat-denatured DNA[c]	(0.0024)	140±20
〃	(0.0056)	105±20
〃	(0.01)	15±5
〃	(0.06)	5±0.5

a) The microtiter plates were coated with 50 ng of native 4NQO-DNA (0.6% modified bases) and applied with 25 ng of IgG per well.
b) No inhibition detected with 10^5 fmol per assay.
c) Heated in PBS at 90° for 3 min.
d) Values in parenthesis expressed as percent of modification level.
e) Values expressed as fmol of 4NQO derivatives.
f) Heated in 0.1*N* NaOH at 90° for 15 min, and precipitated with ethanol.

cross-reactivity to the unmodified denatured DNA (25 μg/well). The 50% inhibition value of the antibodies for the DNAs and related compounds are shown in Table I. As the modification levels were decreased from 0.06% to 0.0024%, the 50% inhibition values increased approximately 30-fold (Table I). The higher 50% inhibition values at low modification levels were not due to interference by the higher concentrations of DNA present in wells compared to assays using highly modified DNA. The reason is that addition of an unmodified denatured DNA (up to 50 μg/well) had no significant effect on competitive ELISA for 0.6% adducted DNA (data not shown).

The antibodies were further characterized as regards cross-reactivity to other carcinogen-modified DNAs, as well as free 4NQO, 4HAQO and 4AQO. They show no cross-reactivity with up to 10^5 fmol of free 4NQO, 4HAQO or 4AQO. DNA modified by other carcinogens, e.g., N-hydroxyacetylaminofluorene or 2,3-dimethyl-4-hydroxyaminopyridine 1-oxide, also did not react with these antibodies even when tested with 10^5 fmol of adducts.

To test the stability of 4NQO adducts to heat denaturation, 4NQO-DNA was assayed by competitive ELISA before and after heat denaturation. The amounts of adducts giving 50% inhibition for 4NQO-DNA containing 0.6% modified bases were 20 fmol before denaturation, 5 fmol after heat denaturation and 2 fmol after alkaline heat treatment. Heating at 70° in 0.01*N* HCl for 15 min reduced the immunological reactivity by 90%. This is consistent with the antibody recognizing predominantly 4NQO adducts on purines. After treatment of 4NQO-DNA in 0.1*N* NaOH at 90° for 15 min, 60% of the 4NQO adducts were lost. Chromatographic analysis of alkali-treated DNA indicated that most of the QGII fraction had disappeared, while QGIII (Guo-N^2-AQO) and adenine adducts remained almost in their entirety (data not shown). As shown in Table I, these antibodies reacted with 4NQO-poly A to a lesser extent than with 4NQO-poly G. From these results, we concluded that the antibodies could react most efficiently with QGIII. However, the possibility that other adducts are also involved has not been ruled out.

Immunofluorescence Studies Indirect immunofluorescence studies indicated that the antibodies specifically bound to cell nuclei treated with various concentrations of 4NQO (Fig. 4). Positive immunofluorescence staining in nuclei could be observed only after treatment with 2.5*N* HCl for 45 min. It is likely that the acid treatment of cells improves the accessibility of the adducts to the antibodies by removal of chromosomal proteins. As the control, non-immune rabbit serum was applied to 4NQO-treated cells or anti 4NQO-DNA IgG to cells which had not been treated with 4NQO. The immunofluorescence was not observed in any of the control cells. With the exception of the nucleoli, specific binding of the anti-4NQO-DNA antibodies is restricted to the nuclei, which exhibit a uniformly distributed fluores-

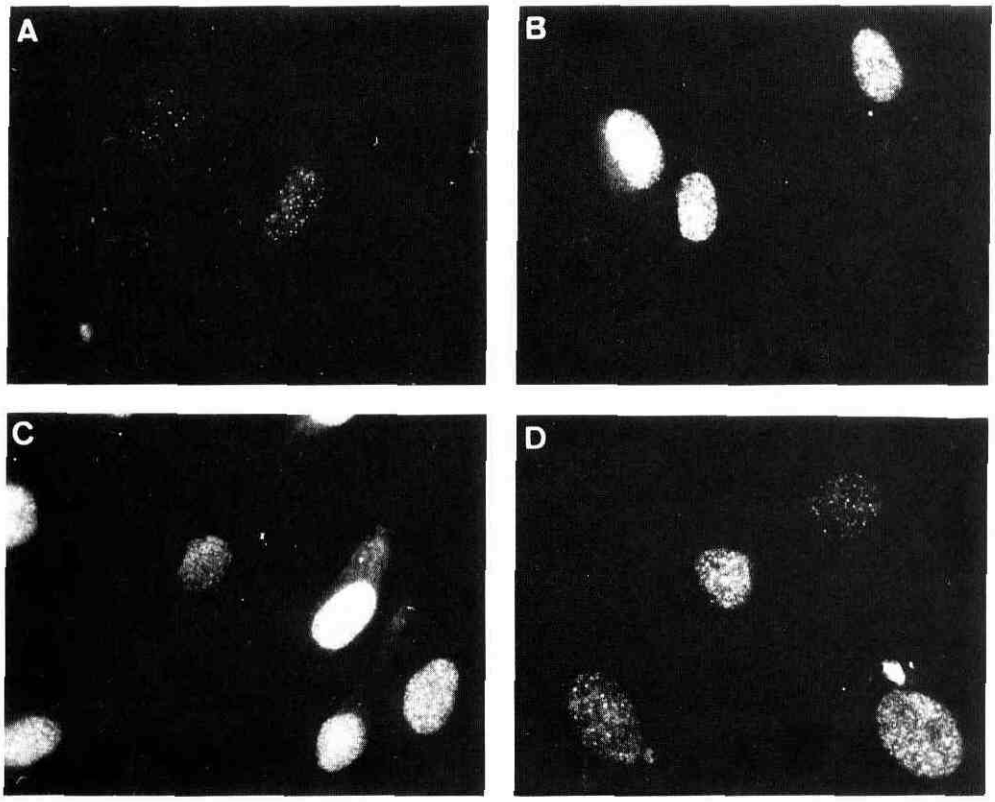

Fig. 4. Specific binding of anti-4NQO-DNA to nuclei of human skin fibroblast cells demonstrated by immunofluorescence. Cells were exposed to the following concentrations of 4NQO. A, 0.25μM; B, 0.5μM; C, 1μM; D as C but then incubated for 16 hr in fresh medium.

cence. As shown in Figs. 4A and 4C, the intensity of immunostaining fluorescence seems to depend on the concentration of 4NQO to which the cells were exposed. To determine the amount of adducts present in the treated cells, DNA was isolated from [^{3}H]-4NQO-treated cells and purified by hydroxyapatite column chromatography,[24] then applied to the radioactivity assay and the competitive ELISA. According to these methods, DNA of the cells treated with 5μM 4NQO contained 10^5 adducts per cell, e.g., 2 adducts per 10^5 nucleotides. Weak fluorescence could be detected with 0.25μM doses, (survival 50%)[25] in this case, producing less than 10^4 adducts per cell. The possibility that the repair of the damaged DNA can be followed and visualized at the single cell level has been examined. In repair experiments, the cells were treated for 60 min with 1μM 4NQO, and examined by indirect immunofluorescence at 16 hr after removal of the 4NQO. The results, that the intensity of the nuclear fluorescence in 4NQO-treated cells was diminished, indicated the possibility of visualizing repair processes *in situ*. Experiments to quantify adduct formation *in situ* by fluorescence intensity measurement are in progress.

Quantification of *in vivo* Adducted 4NQO-DNA in Rat Tissue [^{3}H]4HAQO was administered to rats at the dosage of 20 mg per kg body weight. The modified DNA was isolated from the liver, pancreas, kidney, lung, stomach and uterus after one hour. The DNA was heated at 90° for 3 min in PBS and subjected to competitive ELISA as described in "Materials and Methods." A calibration curve was constructed by pre-incubating 25 ng of IgG fraction with 25 μg of DNA containing 4NQO adducts in the range from 0.8

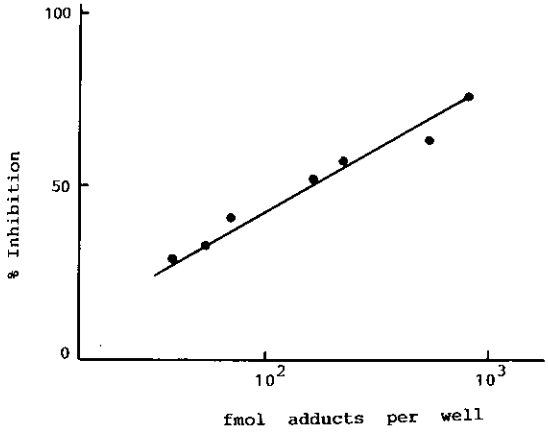

Fig. 5. Competitive inhibition of anti 4NQO-DNA antibodies binding to 4NQO-DNA. Preincubation mixtures (per well) consisted of 25 ng of the antibody and 25 μg of 4NQO-DNA as an inhibitor, which contained 0.8 to 10.9 adducts per 10^6 nucleotides. After preincubation, the mixtures were applied to ELISA.

Table II. Quantitation of [^{3}H]4NQO Binding to Rat Tissues DNA as Determined by Radioactivity Measurements and Competitive ELISA

	Amount of [^{3}H]4NQO bound to DNA (adducts/10^6 nucleotides)	
	Radioactivity	ELISA[a]
Pancreas	117.6	112.0
Kidney	69.6	66.0
Stomach	66.3	62.0
Uterus	65.0	62.5
Lung	26.3	24.2
Liver	1.2	<2.2

a) The percentage inhibition was used to calculate the number of adducts in the samples by comparison with the standard inhibition curve obtained in the same experiments (cf. Fig. 5). The data given here are the averages of two separate DNA isolations.

to 10.9 adducts per 10^6 nucleotides. When DNA of low modificaion, such as biological samples, was examined by competitive ELISA using highly modified 4NQO-DNA as a standard, values measured by ELISA were 30–60% of those obtained from the radioactivity (data not shown). These results might be caused by an ability of anti 4NQO-DNA antibodies, to recognize highly modified DNA more efficiently than DNA modified to a low extent (Table I and Fig. 3). The standard inhibition curve gave 50% inhibition at 170 fmol (see Fig. 5). The percentage of inhibition of antibody binding was calculated for each sample. By comparing the result with the calibration curve, the amount of 4NQO-modified bases could be determined. The results, expressed as the number of 4NQO-modified bases per 10^6 nucleotides, are given in Table II. These values are in the same range as those obtained when measuring the extent of covalent binding to DNA from the radioactivity.

Discussion

The present report describes the production of polyclonal antibodies against 4NQO-DNA. The antibodies obtained are highly specific for 4NQO-DNA, recognizing neither the free quinoline derivatives nor the unmodified DNA. The difference in reactivities found in the direct ELISA between native and denatured DNA at low modification levels and the disappearance of this difference at high modification levels suggested a denaturing effect of adduct formation on DNA structure. Direct evidence of 4NQO binding causing local denaturation has been obtained in studies on the optical melting profiles of DNA treated in the double-stranded form with different amounts of 4NQO.[26, 27] Decrease in the melting temperature and broadening of the transition width were observed, which suggested that the binding sites are distributed randomly along a DNA molecule and result in distortion of the secondary structure. The unwinding of DNA induced by formation of 4NQO adducts at levels of more than 1 adduct per 10^2 nucleotides has been also observed as altered electrophoretic mobility of supercoiled pBR322 DNA treated with 4NQO (Tada *et al.*, unpublished data). These data are consistent with the results obtained for other carcinogens, such as acetylaminofluorene,[28] benzo[*a*]pyrene,[29] and *cis*-diaminodichloroplatinum (II).[30]

When 4 NQO-DNA was digested with nuclease P_1 to mononucleotides, antibodies showed lower reactivity than with non-digested DNA. These results may indicate that these antibodies recognize not only 4NQO adducts themselves, but also their surrounding DNA structure. These results are in

contrast to those obtained with antibodies to N-guanyl-2-acetylaminofluorene, in which modified DNA's reacted less efficiently than hydrolyzed DNA,[31] but are consistent with data on antibodies against benzo[*a*]pyrene[22] or aflatoxin B_1-modified DNA's.[16]

Immunofluorescence studies allow detection of the localization and examination of the formation and removal of the adducts at the single-cell level.[32] It is known that 4NQO covalently binds with DNA, and also proteins and RNA. Since these antibodies reacted with 4NQO-modified RNA, though to a lesser extent than with DNA, the cellular RNA must be degraded with RNase in order to detect only the DNA adduct. Our antibodies are sensitive enough to detect DNA modification levels which allow for an appreciable survival rate of 4NQO-treated human fibroblast cells.[25]

The immunochemical detection system was applied to the analysis of 4NQO-adduct formation in 4NQO-treated rats and compared with the use of radio-labeled adducts. The competitive ELISA reported here is sensitive enough to determine adducts in biological specimens from rats exposed to 4NQO. Such immunochemical and immunohistochemical studies will be useful not only for the detection and quantification of carcinogen-DNA damage, but also for probing aspects of DNA structure and conformation at local sites of carcinogen-DNA damage.

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