In vivo Interaction of 4-Nitroquinoline-1-oxide and its Derivatives with DNA

4-NITROQUINOLINE-1-OXIDE (4NQO) is a highly carcinogenic compound¹. The reductive pathway, which yields 4-hydroxylaminoquinoline-1-oxide (4HAQO), was thought to be essential for its carcinogenic mechanism^{2,3}. There have been reports of the interaction of 4NQO or 4HAQO with DNA *in vitro*⁴⁻⁶ and *in vivo*⁷. This communication deals with the correlation between carcinogenicity and *in vivo* interaction with DNA by 4NQO derivatives.

4NQO or its derivatives (1 mg/rat) was injected intraperitoneally into Donryu rats bearing Yoshida ascites hepatoma AH 130. One hour later, the hepatoma cells were collected and DNA was extracted by the method of Kay *et al.*⁸.

Fluorescence with maxima at 465–475 mμ when excited at 365 mμ was observed in DNA isolated from hepatoma cells of rats injected with carcinogenic 4NQO, 4HAQO or 6-chloro-4-nitroquinoline-1-oxide (6-Cl-4NQO), but not from hepatoma cells of rats injected with non-carcinogenic 4-aminoquinoline-1-oxide (4AQO) or 3-methyl-4-nitroquinoline-1-oxide (3-CH₃-4NQO). The radioactivity in DNA isolated from hepatoma cells of rats injected with 1 mg of ¹⁴C-4NQO (3 × 10⁶ c.p.m./mg) shows that 1.7 moles of quinoline compound was bound with a mole of DNA, if the molecular weight of DNA is 6.7 × 10⁶ (Table 1).

The fluorescent compound in DNA was not eliminated from single strand DNA by heating and rapidly cooling native DNA (Table 1), which suggests that there is a covalent bond between the quinoline compound and the DNA molecule.

Table 1. FLUORESCENCES OF NATIVE AND HEAT-DENATURED DNAs FROM AH 130 TREATED *in vivo* WITH 4NQO AND ITS DERIVATIVES

Injected	Carcinogenicity	Fluorescence (relative intensity/E ₂₆₀)	
		Native	Heat-denatured
4NQO	+	10.4	10.1*
4NQO	+	14.3	13.3†
¹⁴ C-4NQO	+	11.8	—
4HAQO	+	15.3	15.8*
6-Cl-4NQO	+	19.0	—
4AQO	—	0	0*
3-CH ₃ -4NQO	—	0	—
Saline	—	0	0*

* DNAs isolated by ethanol precipitation.

† DNAs isolated on a column of 'Sephadex G-25'.

The reductive pathway to yield 4-hydroxylamino derivatives seems to be necessary to form fluorescent compound in DNA and exert carcinogenic action, because 3-CH₃-4NQO, which cannot be reduced by an enzyme, did not produce fluorescent compound. The more reduced compound 4AQO, which is not carcinogenic, did not produce fluorescence in DNA.

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Isonicotinic Acid Hydrazide as an Inhibitor of Transpeptidation: Relevance for Blood Coagulation

WE have found that isonicotinic acid hydrazide, a widely used drug¹, is a potent inhibitor of the crosslinking of fibrin. This reaction constitutes the terminal step in normal blood coagulation and proceeds by way of an enzyme catalysed transpeptidation by forming inter-fibrin γ-glutamyl-ε-lysine bonds^{2,3}. Compounds which resemble the donor (amine) or the acceptor (carbonyl) side-chain functions of fibrin specifically inhibit the crosslinking reaction without, however, interfering with gel formation—that is, clotting time—itsself.

Table 1

Amine tracer	Type of label and ref.
Glycine ethyl ester	Carbon-14 isotope ^{4,5}
Hydroxylamine	Chemical ⁵
Hydrazine	Chemical ⁵
N-(5-aminopentyl)-5-dimethylamino-1-naphthalenesulphonamide	Fluorescence ³

The compounds listed are sufficiently different in the basicity (nucleophilicity) of amines and in the substituent groups to permit the conclusion that most, if not all, amine inhibitors (H₂N.R) would block the crosslinking sites of fibrin in a similar manner

where reaction (2) competes with the crosslinking process (1), shown for dimerization by a single amide bond (F and F' represent fibrin; ε stands for the crosslinking enzyme, that is, the thrombin-activated fibrin stabilizing factor^{4,7}; Y is the leaving group in transpeptidation).

Highly potent inhibitors were found among primary amines, aminoxy compounds and hydrazine derivatives⁴. Apart from usefulness in elucidating the mechanism of crosslinking, such inhibitors may be of pharmacological significance. Thrombi formed in the presence of these compounds are very much more susceptible to attack by lytic enzymes⁸.

Details are available of the mode of action of the donor type of inhibitors. Four of these (Table 1) were shown to serve as substrates for the crosslinking enzyme. As such, they could be incorporated into fibrin and were, in fact, indispensable for determining the nature of acceptor and donor sites on the protein.

Isonicotinic acid hydrazide

is the most potent inhibitor found so far. In the isolated bovine fibrin crosslinking test system (Fig. 1), its inhibitory effect may be readily demonstrated even in concentrations close to those obtained in plasma during the pharmacological administration of the drug¹.

Fig. 1. Inhibition of fibrin crosslinking by isonicotinic acid hydrazide (Calbiochem, lot 105421). Ordinate denotes the amount of fibrin which cannot be dissolved in 1 per cent monochloroacetic acid. For detailed methodology see refs. 4 and 14.