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Inhibition of aminoazo dye binding to rat liver protein by anthraquinone derivatives

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THE BINDING of the carcinogenic aminoazodye 3′-methyl-4-dimethylaminoazobenzene (3′-MeDAB) to rat liver protein *in vivo* was found to be partially inhibited by intraperitoneal injections of sodium salicylate or of another *ortho*-hydroxybenzoate, aluminon.[1] It seemed possible that the metal chelating properties of the orthohydroxybenzoic acid grouping might play some part in the suppression of aminoazodye binding. Therefore it was of interest to examine the effect of other chelating agents on dye binding.

Among compounds chosen for study were 1-hydroxyanthraquinone and 1-hydroxy-2-methylanthraquinone in which the 1-hydroxy-9-oxo configuration has chelating potentiality. These compounds caused some reduction in the amount of bound dye produced by 3′-MeDAB in rat liver. However, the parent substance anthraquinone was just as effective and all of these anthraquinones were less effective than 2-methylanthraquinone and 1-amino-2-methylanthraquinone which reduced dye binding by more than 50 per cent.

Anthraquinone also suppressed the increase in rat liver GSH content due to 3′-MeDAB. Control experiments revealed that all the anthraquinone derivatives with one exception tended to increase the glutathione (GSH) content of male rat liver, the most effective being 1,2-benzanthraquinone.

For comparison, the effect of 2-methylnaphthoquinone on male rat liver bound dye (B.D.) and GSH content was examined and a brief study was made of the effect of two polycyclic aromatic hydrocarbons on the same parameters.

EXPERIMENTAL

Adult stock male albino rats (250–350 g body wt.) were maintained on water and Oxoid Diet 86 (made by the Oxoid Division of Oxo Ltd., London). From 10 to 11 a.m., pairs of rats were injected intraperitoneally with 3′-MeDAB in arachis oil (8·25 mg of 3′-MeDAB/0·6 ml per 100 g body wt.) or with arachis oil only (0·6 ml/100 g body wt.). Other rats received the same injections incorporating the test anthraquinone derivative as a fine suspension or solution in the oil. The anthraquinones as well as 2-methylnaphthoquinone and the polycyclic aromatic hydrocarbons were all given at a dose equivalent on a mole for mole basis to the standard dose of 8·25 mg of 3′-MeDAB/100 g body wt.

Anthraquinone and 1-hydroxyanthraquinone (Koch-Light Laboratories, Ltd.) were recrystallized from ethanol. 1,2-Benzanthraquinone was a commercial sample which was not recrystallized. 1-hydroxy-2-methylanthraquinone (m.p. 182–183°) was prepared by the method of Marschalk *et al.*[2] 2-methylanthraquinone (m.p. 171°) was prepared according to Fieser.[3] 1-Nitro-2-methylanthraquinone was prepared by nitration of 2-methylanthraquinone and was reduced to 1-amino-2-methylanthraquinone (red needles, m.p. 199–200°).[4] 2-Methylnaphthaquinone was obtained from British Drug Houses, Ltd, 20-methylcholanthrene from Roche Products and 9,10-dimethyl-1,2-benzanthracene from Koch-Light Laboratories.

In each experiment, one pair of 3′-MeDAB-injected rats and one pair of arachis oil-injected control rats were included. Each anthraquinone derivative and 2-methylnaphthoquinone was tested in two pairs of rats. Only one pair of rats was used for each of the hydrocarbons examined.

The rats were killed with ether about 24 hr after injection and the livers were perfused with ice-cold normal saline and stored immediately in solid carbon dioxide. B.D. and GSH estimations were carried out as already described[1] on liver powders and trichloracetic acid extracts derived from pooled 1 g samples of frozen liver from each of a pair of identically-treated rats.

RESULTS

B.D. and GSH estimations are given in the accompanying Table which was compiled from the results of eight separate experiments.

Each anthraquinone derivative partially inhibited 3′-MeDAB dye binding in the order of increasing activity: 1,2-benzanthraquinone, 1-hydroxyanthraquinone, anthraquinone, 1-amino-2-methylanthraquinone and 2-methylanthraquinone. Only anthraquinone exerted a definite suppression of the GSH content of livers of rats injected with 3′-MeDAB.

TABLE 1. EFFECT OF ANTHRAQUINONE DERIVATIVES AND OTHER SUBSTANCES ON THE B.D. AND GSH CONTENT OF LIVERS OF MALE RATS INJECTED WITH 3′-MeDAB IN ARACHIS OIL OR WITH ARACHIS OIL ONLY

Substance included in main injection	Main injection					
	No. of pairs of rats	3′-MeDAB in arachis oil		No. of pairs of rats	Arachis oil only	
		B.D. *E* at 520 mμ	GSH mg (%)		B.D. *E* at 520 mμ	GSH mg (%)
Nil	8	0·135 ± 0·004*	286·9 ± 9·0*	8	0·015 ± 0·002*	171·7 ± 5·2*
2-Methyl AQ†	2	0·047, 0·058	279·3, 264·5	2	0·018, 0·027	184·0, 240·0
1-Hydroxy-2-methyl AQ	2	0·077, 0·080	253·8, 267·0	2	0·024, 0·027	192·0, 209·3
1-Hydroxy AQ	2	0·078, 0·088	235·2, 281·8	2	0·030, —	200·9, 210·7
1-Amino-2-methyl AQ	2	0·045, 0·064	247·2, 250·7	2	0·013, —	181·0, 188·0
AQ	2	0·075, 0·076	227·5, 244·8	2	0·028, —	182·4, 216·8
1,2-BenzAQ	2	0·080, 0·109	289·1, 322·0	2	0·007, 0·015	203·4, 228·0
2-Methylnaphthoquinone	2	0·076, 0·033	225·4, 143·4	2	— , 0·005	139·4, 141·6
20-Methylcholanthrene	1	0·094	244·8	1	0·016	188·0
9,10-Dimethyl-1,2-benzanthracene	1	0·119	305·9	1	0·016	177·1

* Mean ± standard error.
† AQ—Anthraquinone.
— Not done.

With the exception of 1-amino-2-methylanthraquinone the other anthraquinone derivatives showed some activity in increasing the GSH content of normal rat liver, 1,2-benzanthraquinone being most active in this respect.

2-Methylnaphthoquinone appeared to be quite toxic. There was much ascites fluid and the livers were shrunken after injections of this quinone. The organs were stiff and there were strong adhesions of stomach and kidney to liver. The compound inhibited dye binding and also decreased considerably the GSH content of both normal and 3′-MeDAB-treated rat livers. GSH is known to unite through its thiol group at the 3-position of 2-methylnaphthoquinone *in vitro*.[5] It seems possible that the marked decline in liver GSH content may be attributed in part to this reaction.

Of the two polycyclic hydrocarbons examined, 20-methylcholanthrene inhibited dye binding in agreement with the findings of Miller *et al.*[6] 9,10-dimethyl-1,2-benzanthracene affected neither the B.D. nor the GSH content of the livers of 3′-MeDAB-injected rats. Injection of the hydrocarbons by themselves had no effect on the GSH content of normal rat liver.

DISCUSSION

Although information is lacking on the metabolic transformations of the anthraquinone derivatives which we have studied, it would appear that the presence of the 1-hydroxy-9-oxo grouping is not essential for the inhibition of 3′-MeDAB dye binding by these compounds. In unpublished work it was found that other chelating agents such as 8-hydroxyquinoline and 2,9-dimethyl-1,10-phenanthroline had no effect on the amount of B.D. or liver GSH increase due to 3′-MeDAB.

It is of interest that some of the anthraquinone derivatives have the ability to increase the liver GSH content of normal male rats. This effect has been observed following the injection of a number of hepatocarcinogenic agents including aminoazo dyes,[7] 2-acetylaminofluorene and DL-ethionine.[8] It might be worth examining anthraquinone derivatives for hepatocarcinogenic activity particularly as there is some evidence already that 1,8,9-anthratriol has cocarcinogenic activity,[9] that anthraquinone has weak carcinogenic activity[10] and that certain anthraquinonoid metabolites of *Penicillium slandicum* (e.g. luteoskyrin, a compound containing two hydroxylated 1-hydroxy-2-methylanthraquinone residues) may be the active hepatocarcinogenic agents in yellow mouldy rice.[11]

SUMMARY

Anthraquinone derivatives partially inhibited the binding of 3′-methyl-4-dimethylaminoazobenzene to rat liver protein. Some of these derivatives increased the glutathione content of normal male rat liver.

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