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EFFECT OF α-TOCOPHERYL ACETATE ON LIVER GLUTATHIONE OF MALE RATS INJECTED WITH 3′-METHYL-4-DIMETHYLAMINOAZOBENZENE

W. J. P. NEISH and ANN RYLETT

Cancer Research Unit, University of Sheffield, Sheffield, 10, England

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Abstract—The effect of D- and of DL-α-tocopheryl acetate on the ability of the carcinogenic azo dye, 3′-methyl-4-dimethylaminoazobenzene, to increase male rat liver glutathione has been examined.

Tocopheryl acetate suppresses to some extent the increase in liver glutathione due to the azo carcinogen, and, at an optimal dose, prevents dilation of the stomach which is found in rats injected 24 hr previously with 3′-methyl-4-dimethylaminoazobenzene.

LIVERS of male albino rats injected 24 hr previously with a solution of the hepatocarcinogenic azo dye 3′-methyl-4-dimethylaminoazobenzene (3′-MeDAB) in arachis oil were found to contain twice as much glutathione (GSH) as livers of control rats injected intraperitoneally with arachis oil.[1]

Recently, Ryerson *et al.*[2] showed that the levels of liver GSH are higher in Vitamin E deficient rabbits than in normal rabbits. GSH levels appear to be controlled in some way by Vitamin E.

It was of interest to determine to what extent if any the increase in rat liver GSH is due to a possible depletion of liver Vitamin E stores through the action of the carcinogen. We have now investigated the effect of supplements of α-tocopheryl acetate (TA) on the GSH-inducing ability of 3′-MeDAB.

EXPERIMENTAL

Pairs of stock adult male albino rats (~300 g body weight) were given intraperitoneal injections of 3′-MeDAB in arachis oil (16·5 mg 3′-MeDAB in 0·6 ml arachis oil per 100 g body weight) or of similar solutions to which had been added 20 or 100 mg of D-α-tocopheryl acetate (D-TA; Light and Co.) or of α-tocopheryl acetate (presumably the synthetic DL-form), which was obtained by ether extraction of a commercial Vitamin E acetate tablet preparation—Ephynal (Roche). Pairs of control rats received injections of arachis oil (0·6 ml per 100 g body weight) or of arachis oil plus D-TA or DL-TA. Azo dye and TA supplements were dissolved in the appropriate amount of warm arachis oil immediately before injection. When 100 mg supplements of TA were given, 0·1 ml arachis oil was omitted from the injection mixture.

The rats, which had access to water and Diet No. 86 *ad libitum*, were killed 24 hr after injection and the perfused livers were frozen at once in solid carbon dioxide. GSH determinations were carried out on pooled trichloracetic acid (TCA) extracts of 5 g samples of livers of pairs of identically treated rats by the cuprous mercaptide method already described.[1] In four separate experiments, the livers were collected as

rapidly as possible at the same time of day (11 a.m.–12 noon). In each experiment, recovery experiments were run on 10 and 15 mg samples of authentic GSH. For the experiments described as A, B, C and D in Tables 1 and 2, GSH recoveries were respectively 65·0, 64·0, 63·1, 70·4; 68·1, 75·0 and 64·3, 56·4 per cent.

RESULTS AND DISCUSSION

GSH levels of livers of rats which received injections of 3′-MeDAB in arachis oil with or without supplements of D-TA or DL-TA and of livers of corresponding control rats are given in Tables 1 and 2. The effects of TA on a dilated condition of the stomachs of rats injected with 3′-MeDAB are also noted.

It is convenient to summarise the effect of TA supplements on the liver GSH levels of 3′-MeDAB-treated rats and on the appearance of stomachs as follows:

Dose of TA	Effect of TA on liver GSH level due to 3′-MeDAB. Change in level		Effect of TA on stomach dilation due to 3′-MeDAB.
	mg	%	
20 mg DL-TA	−32·5	14·6	none
20 mg D-TA	−30·1	14·9	prevents
100 mg DL-TA	−31·7	14·0	prevents
100 mg D-TA	−33·3	14·3	none
100 mg D-TA	nil	nil	none
200 mg DL-TA	not examined		none

Doses of TA from 20 mg of the DL-isomer to 100 mg of D-isomer suppressed the elevated level of liver GSH due to 3′-MeDAB by a constant amount. However, in another experiment with 100 mg doses of D-TA (Table 1, B) no suppression was obtained. There may be a critical dose of about 100 mg D-TA at or above which the partial suppressive action of the vitamin on 3′-MeDAB-induced GSH is abolished. There is an optimal dose of about 20 mg D-TA to 100 mg DL-TA which is capable of preventing stomach dilation due to 3′-MeDAB.

Doses of TA which can produce a 14 per cent depression in the GSH levels of livers of 3′-MeDAB-injected rats might be expected to be equally effective in depressing the GSH content of normal rat liver. This was not so. No suppression at all was noted in the D-TA experiment (Table 1, A). In the DL-TA experiments (Table 2) a suppression of about 17 per cent was observed in experiment C. However, a suppression of only 6 per cent was noted in experiment D. There appears to be a minimal level of GSH of about 90 mg per cent in normal rat liver which is resistant to any further suppressive action of TA. This suggests that any GSH in excess of 90 mg per cent in normal rat liver may be the result of a Vitamin E deficiency which can be corrected by TA injections.

Although György and Goldblatt[3] noted that tocopherol can afford some protection against rat liver necrosis due to 4-dimethylaminoazobenzene (DAB), Sugiura[4] found that dietary supplements of wheat germ oil (rich in tocopherol) had no effect on the course of tumour production in rat liver by DAB. Similarly when α-tocopherol was fed together with 3′-MeDAB to male rats, the tumour yield was not significantly different from that obtained in the absence of tocopherol.[5]

Elevation of rat liver GSH may be an important factor for hepatocarcinogenisis by 3′-MeDAB.[1] If this is true, tocopheryl acetate supplements which can suppress by only 14 per cent the rise in rat liver GSH due to 3′-MeDAB would not be expected to modify profoundly the course of 3′-MeDAB carcinogenesis.

TABLE 1. EFFECT OF D-α-TOCOPHERYL ACETATE (D-TA) ON MEAN GSH CONTENT OF LIVERS OF PAIRS OF MALE ALBINO RATS 24 HR AFTER INTRAPERITONEAL INJECTION OF 3′MEDAB IN ARACHIS OIL (AO) OR WITH ARACHIS OIL ONLY

Experiment	Weight of rats at time of injection (g)	Injection	GSH level mg/100 g liver wet weight	Condition of stomachs
A	355 280	3′-MeDAB in AO	202·0	very dilated
	329 364	3′-MeDAB in AO + 20 mg D-TA	171·9	normal
	355 356	3′MeDAB in AO + 100 mg D-TA	202·0	very dilated
	275 370	AO	90·6	normal
	375 305	AO + 20 mg D-TA	94·5	normal
	358 395	AO + 100 mg D-TA	90·6	normal
B	280 250	3′-MeDAB in AO	233·0	very dilated
	280 300	3′-MeDAB in AO + 100 mg D-TA	199·7	very dilated
	315 372	AO	116·9	normal

TABLE 2. EFFECT OF DL-α-TOCOPHERYL ACETATE (DL-TA) ON MEAN GSH CONTENT OF LIVERS OF PAIRS OF MALE ALBINO RATS 24 HR AFTER INTRAPERITONEAL INJECTION OF 3′-MEDAB IN ARACHIS OIL (AO) OR WITH ARACHIS OIL ONLY

Experiment	Weight of rats at time of injection (g)	Injection	GSH level mg/100 g liver wet weight	Condition of stomachs
C	325 305	3′-MeDAB in AO	222·9	very dilated
	315 336	3′-MeDAB in AO + 20 mg DL-TA	190·4	very dilated
	348 309	AO	107·6	normal
	310 342	AO + 20 mg DL-TA	89·0	normal
D	339 308	3′MeDAB in AO	226·0	very dilated
	315 305	3′-MeDAB in AO + 100 mg DL-TA	194·3	normal
	340 311	AO	99·1	normal
	312 296	AO + 100 mg DL-TA	92·9	normal

Stomach dilation* which is observed in rats 24 hr after 3′-MeDAB injection may result from a temporary constriction of the pyloric sphincter. The stomach is filled with food (and faecal matter?), while the small intestine is practically empty. The blockage is released naturally (it has never been observed in rats at 48, 72 or 96 hr after 3′-meDAB injection) or it can be prevented by an optimal dose of D-TA or DL-TA. Interference with digestive processes might lead to a disturbance in liver metabolism. However as far as the liver GSH level is concerned, the presence (experiment A, 20 mg DL-TA) or absence (experiment B, 100 mg DL-TA) of stomach dilation has not interfered with the action of 3′-MeDAB.

No marked changes in the pattern of ninhydrin-positive components could be detected in two-dimensional chromatograms of liver extracts of 3′-MeDAB and control rats as the result of injection of TA supplements. In the TCA 10%-precipitated proteins of 3′-MeDAB livers, the intensity of the pink colour of the precipitates (due to bound azo dye) was not detectably altered when TA supplements were given.

Factors other than Vitamin E deficiency must be involved in the marked elevation of rat liver GSH due to 3′-MeDAB. Increases in rat liver GSH have been observed in conditions of thiamine[6] or Vitamin B_6 deficiency.[7] Thyroidectomy[8] (and/or thiouracil injections) leads to an increase in the level of free sulphydryl of rat liver.

* Dilation has not been observed after 24 hr in rats which had been injected with $\frac{1}{4}$ of the standard dose of 3′-MeDAB. It was sometimes present after injection of $\frac{1}{2}$ the standard dose. With the standard dose, marked dilation was always observed in either male or female rats. A similar dilation of the stomach has been seen in male rats at 4 and 24 hr after injection of an aqueous solution of sodium salicylate (50 mg salicylate per 100 g body weight).

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