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SHORT COMMUNICATIONS

Effect of tocotrienol on the activities of cytosolic glutathione-dependent enzymes in rats treated with 2-acetylaminofluorene

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Abstract—The effect of tocotrienol on the activities of glutathione *S*-transferases (GSTs), glutathione reductase (GR) and glutathione peroxidase (GPx) in rats given 2-acetylaminofluorene (AAF) was investigated over a 20 week period. Liver and kidney GST and liver GR activities were significantly increased after AAF administration. Kidney GPx activities were significantly affected; activity assayed with cumene hydroperoxide (cu-OOH) was increased but activity assayed with H_2O_2 was reduced. Supplementation of the diet with tocotrienol in the AAF-treated rats reduced the increase in enzyme activities. Tocotrienol on its own had no effect on the enzyme activities.

A number of glutathione (GSH*)-dependent enzymes notably glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione *S*-transferases (GSTs) have been reported to correlate closely with the occurrence and stages of neoplasia [1, 2]. It has been reported that the severity of carcinogenesis is moderated by vitamin E [3]. Tocotrienol, a form of vitamin E, has been reported to exhibit antitumour activity against several transplantable murine tumours [4]. It was also reported that tocotrienol reduces the severity of hepatocarcinogenesis in the rat induced by 2-acetylaminofluorene (AAF) [5]. We report here the effect of tocotrienol purified from palm oil on the activities of GSTs, GPx and GR in the livers, lungs and kidneys of rats treated with AAF.

Methods and Results

Animal treatment was carried out essentially like Wan Ngah *et al.* [5] except that the animals were killed at 3, 8, 12 and 20 weeks. The liver, lungs and kidneys were then removed and weighed, and cytosolic enzyme extraction was carried out following the procedure of Speier and Wattenberg [6].

GST activities were assayed following the method of Habig *et al.* [7] using 1-chloro-2,4-dinitrobenzene (CDNB) and 3,4-dichloro-nitrobenzene (DCNB) as the second substrates. GR activity was assayed according to the method of Racker [8]. GPx activity was assayed spectrophotometrically according to the method of Paglia and Valentine [9] with slight modifications; to 0.8 mL of the reaction mixture containing 55 mM phosphate buffer pH 7.0, 0.84 mM NADPH, 11.25 mM NaN_3 and 0.5 mM reduced GSH, 0.1 mL of the cytosolic fraction was added. The reaction was started by the addition of 0.1 mL 2.5 mM H_2O_2 or 0.1 mL 2.2 mM cumene hydroperoxide (cu-OOH).

The protein concentrations of the cytosolic fractions were determined by the method of Sedmark and Grossberg [10]. The results obtained in the experiment (Table 1) were analysed by Student's *t*-test and the value of $P < 0.05$ was considered as significant.

AAF treatment significantly increased liver and kidney GST and liver GR activities. Kidney GPx (cu-OOH) activity was increased while GPx (H_2O_2) activity was reduced. Liver GPx and kidney GST (CDNB) and GR activities, as well as all the enzyme activities in the lung, were not affected. The enzyme activities in the treated organs were significantly affected 3 weeks after administration of AAF or the AAF–tocotrienol mixture and remained elevated for up to 20 weeks. Tocotrienol moderated the increase in the enzyme activities brought about by AAF. However, on its own it had no effect on any of the enzyme activities determined in all the organs tested of normal rats (data not shown).

Discussion

The GSH-dependent enzymes, especially the GSTs, have been reviewed in relation to carcinogenesis [11, 12]. The GST placental form (form 7), a marker for neoplasia [2, 13–15], is induced in the liver by carcinogens and contributes to the increase in overall GST levels in hepatocarcinogenesis. In the kidney, the increase in GST (DCNB) activity could be due to an increase in one or more of the isoenzyme forms that are different from the GST placental form. The kidney GST isoenzyme form 3 is not normally present in the liver, is specifically very active against DCNB and is distinct from the GST placental form.

Kitahara *et al.* [16] reported that GPx and GR activities were increased in neoplasia. The increase in GPx activity was due to the increased GPx activity of GST [17]. A GST isoenzyme form exhibits GPx (cu-OOH) activity but not GPx (H_2O_2) activity. It is possible that the GPx activity of GST contributes to the total increase in GPx activity in the kidneys of the AAF-treated rats since both GST (DCNB) and GPx (cu-OOH) activities were correspondingly increased.

We have suggested in an earlier report [5] that tocotrienol reduces the severity of hepatocarcinogenesis in AAF-treated rats based on gross morphology, ultrastructural and histological studies coupled with determination of plasma and liver γ-glutamyltranspeptidase and liver UDP-glucuronyltransferase. In the present study, we have shown that tocotrienol supplementation in the diet reduces the increase in GST and GPx activities, both of which have been reported to be closely correlated to the severity of carcinogenesis. This finding provides further evidence for the suggestion that tocotrienol reduces the severity of hepatocarcinogenesis.

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* Abbreviations: GST, glutathione *S*-transferase (EC 2.5.1.18); GR, glutathione reductase (EC 1.6.4.2); GPx, glutathione peroxidase (EC 1.11.1.9); AAF, 2-acetylaminofluorene; cu-OOH, cumene hydroperoxide; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 3,4-dichloronitrobenzene; GSH, glutathione.

Table 1. Effect of AAF and AAF–tocotrienol mixture on cytosolic enzyme specific activities in the liver and kidney of rats

Week	3	8	12	20
Liver GST (CDNB)				
Control	0.71 (0.07)	0.84 (0.02)	0.65 (0.03)	0.50 (0.04)
AAF	0.94 (0.06)*	0.97 (0.12)*	1.10 (0.08)*	0.50 (0.07)
Mixture	0.99 (0.04)*	0.93 (0.03)*	0.89 (0.08)*	0.47 (0.03)
Liver GST (DCNB) (activity $\times 10^{-2}$)				
Control	2.53 (0.21)	3.03 (0.11)	2.43 (0.20)	2.53 (0.42)
AAF	3.60 (0.20)*	3.53 (0.16)*	3.90 (0.29)*	5.37 (0.12)*
Mixture	3.20 (0.21)*	3.30 (0.19)	3.48 (0.31)*	4.72 (0.30)*
Liver GR (activity $\times 10^{-2}$)				
Control	4.93 (0.29)	5.15 (0.15)	5.05 (0.23)	6.10 (0.51)
AAF	6.28 (0.24)*	6.80 (0.42)*	7.17 (0.47)*	9.78 (0.25)*
Mixture	7.25 (0.45)*	6.80 (0.45)*	6.38 (0.57)	8.33 (0.57)*
Kidney GST (DCNB) (activity $\times 10^{-3}$)				
Control	0.42 (0.03)	0.54 (0.06)	0.55 (0.03)	0.63 (0.14)
AAF	0.42 (0.05)	0.93 (0.10)*	1.39 (0.29)*	1.87 (0.22)*
Mixture	0.42 (0.03)	0.90 (0.09)*	1.24 (0.14)*	1.43 (0.10)*
Kidney GPx (cu-OOH)				
Control	0.07 (0.01)	0.15 (0.03)	0.12 (0.01)	0.15 (0.02)
AAF	0.13 (0.01)*	0.23 (0.01)*	0.21 (0.01)*	0.19 (0.01)*
Mixture	0.15 (0.02)*	0.15 (0.03)†	0.21 (0.01)*	0.18 (0.01)*
Kidney GPx (H_2O_2)				
Control	0.10 (0.01)	0.11 (0.03)	0.09 (0.01)	0.10 (0.02)
AAF	0.05 (0.01)*	0.06 (0.01)*	0.06 (0.01)*	0.06 (0.01)*
Mixture	0.11 (0.02)†	0.10 (0.02)†	0.06 (0.01)*	0.05 (0.01)*

Values shown are means with ± SD in parentheses.
Significant values: * $P < 0.05$ compared to control, † $P < 0.05$ compared to AAF.

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