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Effect of Butylated Hydroxytoluene and Paraquat on Urethan Tumorigenesis in Mouse Lung

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The antioxidant butylated hydroxytoluene /BHT/ applied as food preservative and the widely used herbicide, Paraquat /1,1'-dimethyl-4,4'-dipyridylium dichloride/ cause pathological changes in the lungs of mice /MARINO and MITCHELL 1972, CONNING et al. 1969/. Urethan /ethylcarbamate/, one of the characteristic representative of carbamate type compounds occurring in various foods and beverages /OUGH 1976a,b/ may induce tumors in mouse lung /MIRVISH 1968/. Our earlier results suggested that LATI:CFLP mice are highly susceptible to the carcinogenicity of urethan /BOJÁN and DAUDA 1976/. Therefore this stock seemed to be suitable to investigate whether BHT or Paraquat may influence the urethan tumorigenesis in mouse lung.

Materials and Methods

Our experiments were carried out on 30-35 days old female CFLP mice obtained from the Laboratory Animals Breeding Centre /Gödöllő, Hungary/. The BHT /Sigma/ was dissolved in corn oil whereas Paraquat /Alkaloida, Tiszavasvári, Hungary/ and urethan /REANAL, Budapest/ were dissolved in saline. In the preliminary experiments the maximum tolerable dose of BHT and Paraquat was determined. After BHT treatment on 1-8 days and after Paraquat treatment on 1-4 days, the lungs of 3-3 animals were fixed in 10 % formalin and the sections were stained with hematoxylin and eosin. Furthermore, the mice were treated with maximum tolerable doses as well as with their fractions and with a single dose of urethan of 1 mg/g body weight ip. Autopsy was carried out on the 35th day after the urethan treatment, and the subpleural lung tumors were counted by a stereomicroscope /magnification 6.3 x/.

Results and Discussion

The maximum tolerable single dose of BHT administered ip. to CFLP mice was found to be 800 mg/kg. The proliferation of alveolar epithelium in the lungs of mice treated with BHT of 100 mg/kg or higher dose was

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observed. The proliferation frequently was so extensive that the cell growth almost filled the alveolar space. These observations are in good agreement with the results obtained in experiments on other mouse strains /MARINO and MITCHELL 1972/. According to the electronmicroscopic studies of WITSCHI and COTÉ /1976/ the majority of cells stimulated to proliferate by BHT are of II type alveolar cells. The CFLP mice tolerated maximum 20 mg/kg Paraquat given ip., which caused haemorrhage, interstitial inflammation and edema. These damages are moderate in case of 20 mg/kg dose, whereas they become more pronounced when higher doses are administered. CLARK et al. /1966/ and ROBERTSON et al. /1971/ observed similar lung damages in rats treated with Paraquat. After Paraquat treatment, electronmicroscopically mainly the toxic damage of alveolar epithelium was seen, and during regeneration the enhanced mitotic activity of II type alveolar cells was observed /VIJEYARATNAM and CORRIN 1971/.

According to our further experiments both BHT and Paraquat enhance the carcinogenicity of urethan in CFLP mice. The potentiating effect of BHT was found to be significant when the urethan was administered after the BHT by 6 or 7 days later. In this case, the yield of induced lung tumors was increased in proportion to the applied dose of BHT /Table I/. During the preparation of our manuscript, WITSCHI et al. /1977/ reported that BHT enhanced the carcinogenic effect of urethan if BHT was administered weekly after the urethan injection for 9-13 weeks. Paraquat significantly enhanced the number of lung tumors induced by urethan administered 2 days after the Paraquat treatment, and this effect was found to be dose-dependent as well /Table II/.

Lung tumors induced by urethan, histogenetically develop from II type alveolar cells /SHIMKIN and STONER 1975/. Both after BHT and Paraquat treatment, the mitotic activity of II type alveolar cells was increased. These results suggest that BHT and Paraquat enhance the carcinogenicity of urethan by increasing the number of proliferating target cells in the lung. An analogous phenomenon was observed when the hepatocarcinogenic effect of urethan was investigated by POUND and LAWSON /1974/ after partial hepatectomy.

Table I

Lung tumor yield after BHT plus urethan treatment in CFLP mouse

Time of urethan inj., days before/-/ or after/+/ BHT or oil treatment	Treatment	n	Animals with tumors n	Number of tumors n	P_{10}-P_{90}*
- 26	corn oil	13	0.69	1.30	0 - 3
	800 mg/kg BHT	15	0.67	1.33	0 - 3
- 2	corn oil	15	0.60	1.06	0 - 3
	800 mg/kg BHT	23	0.65	1.13	0 - 3
0	corn oil	30	0.66	1.16	0 - 3
	800 mg/kg BHT	53	0.69	1.54	0 - 3
+ 2	corn oil	27	0.70	1.25	0 - 3
	800 mg/kg BHT	20	0.60	1.20	0 - 3
+ 5	corn oil	18	0.77	1.38	0 - 3
	800 mg/kg BHT	15	0.80	2.13	0 - 4
+ 6	corn oil	16	0.62	1.06	0 - 3
	800 mg/kg BHT	32	0.68	2.46**	0 - 7
+ 7	corn oil	33	0.66	1.36	0 - 3
	100 mg/kg BHT	15	0.80	1.73	0 - 4
	200 mg/kg BHT	24	0.83	1.91	0 - 6
	400 mg/kg BHT	56	0.71	2.21**	0 - 6
	800 mg/kg BHT	38	0.78	2.89***	0 - 7
+ 8	corn oil	15	0.66	1.40	0 - 3
	800 mg/kg BHT	19	0.73	2.00	0 - 5
+ 10	corn oil	15	0.66	1.53	0 - 4
	800 mg/kg BHT	27	0.66	1.77	0 - 6
+ 14	corn oil	18	0.72	1.44	0 - 4
	800 mg/kg BHT	16	0.81	1.75	0 - 5
untreated control		20	-	-	-
only 800 mg/kg BHT		17	-	-	-
only 1 mg/g urethan		17	0.70	1.47	0 - 3

*10 and 90 percentile values

**significantly different from animals given corn oil, $p < 0.05$

***$p < 0.01$

Table II

Lung tumor yield after Paraquat plus urethan treatment in CFLP mouse

Time of urethan injection, days after Paraquat treatment	Dose of Paraquat mg/kg	n	Animals with tumors n	Number of tumors n	P_{10}-P_{90}*
0	20	18	0.50	1.16	0 - 3
1	20	18	0.66	1.50	0 - 4
2	5	15	0.80	1.20	0 - 2
	10	28	0.71	1.67	0 - 4
	20	34	0.76	2.32**	0 - 5
3	20	14	0.64	1.21	0 - 3
4	20	21	0.76	1.47	0 - 3
untreated control		20	-	-	-
only 20 mg/kg Paraquat		20	0.05	0.05	-
only 1 mg/g urethan		35	0.62	1.17	0 - 3

*10 and 90 percentile values

**significantly different from animals given only urethan, $p < 0.05$

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