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Promoting Effect of Basic Lead Acetate Administration on the Tumorigenesis of Lung in N-Nitrosodimethylamine-treated Mice

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Lead compounds are frequent environmental contaminations and some of them have been found to be carcinogenic for animals, although epidemiological studies have not been considered to provide sufficient evidence that exposure to lead or lead compounds causes cancer in humans. There are only a few reports on the promoting effect of lead on chemical carcinogenesis in vivo. By the concurrent administration of lead compounds and certain organic carcinogens to rats or hamsters, the cocarcinogenic activity of lead has been found in the kidney of rats (Hiasa et al. 1983) or the lung of hamsters (Kobayashi and Okamoto 1974). Hereafter much more attention should be placed to the promoting effect of lead compounds on chemical carcinogenesis. In this report the promoting effect of posttreatment with basic lead acetate (BLA) on the development of lung tumors in strain dd mice exposed to N-nitrosodimethylamine (NDMA) was examined. The concentration of lead and the activity of γ-glutamyltranspeptidase (γ-GTP) in the lung during the carcinogenicity experiment were also measured.

MATERIALS AND METHODS

Male mice of strain ddy (Matsumoto Labo-Animal Co., Kimitsu, Chiba-ken), weighing 22-24 g at the start of the experiment, were used. The animals were maintained on a basal diet and given water ad libitum. NDMA (Wako Pure Chemicals, Tokyo) was dissolved in saline and BLA (purity: above 99.99%, Rare Metallic Co., Tokyo) was suspended in 50% glycerine solution. Animals for the carcinogenicity experiment were divided into the following groups:Group 1(control group)i.p. received 0.1 ml of saline and 50% glycerine solution as a control. Groups 2 (NDMA, 3 inj) and 3 (NDMA, 2 inj) continuously received

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10 mg NDMA/kg body weight 3 and 2 times once a week, respectively. Group 4 (BLA, 18 inj) continuously received 10 mg lead/kg body weight 18 times once a week. Groups 5 (NDMA, 3 inj + BLA, 18 inj) and 6 (NDMA, 2 inj + BLA, 18 inj) continuously received 10 mg lead/kg body weight 18 times once a week from 1 week after being treated similarly to groups 2 and 3, respectively. Fifteen mice were prepared for group 1 (control group), sixteen mice prepared for each group of groups 2, 3 and 5 and twenty mice prepared for each group of groups 4 and 6. It was only by an accident that mice died during the experiment. All mice were sacrificed 18 weeks after starting the injection of BLA. Organ tissues in each group for carcinogenicity experiment were fixed in 10% buffered formalin for a week and embedded in paraffin. Four μm serial paraffin sections were cut and stained with hematoxylin and eosin (H-E) and by the Masson trichrome method. Mice for determining lead concentration and γ-GTP activity were sacrificed immediately before next administration of lead. Six to eight mice were used for each point. The tissues were weighed, minced and decomposed by the wet ashing method using concentrated nitric acid and hydrogen peroxide. The atomic absorption spectrophotometry as follows. The sample solution (10 μl) after the decomposition was atomized at 1800°C in a graphite atomizer for 10 s, and the absorbance was measured at 283.3 nm. A Hitachi atomic absorption spectrophotometer(170-50A, GA-2) with a graphite furnace was used.
For determining γ-GTP activity, the tissues were weighed, minced, homogenized and centrifuged. The 9,000 g supernatant was used for the determination by the method of Talanko and Ruoslanti (1979).

RESULTS AND DISCUSSION

Animals of groups treated with NDMA alone (groups 2 and 3) or BLA alone (group 4) gained body weight to the same extent as control mice (group 1) during the carcinoigenicity experiment, although animals of groups treated with NDMA plus BLA (groups 5 and 6) gained slightly lower body weight than control mice (group 1). The surviving mice were sacrificed 18 weeks after the first injection of BLA, 11 of 14 mice had developed lung tumors in the group (group 2) treated with NDMA (three i.p. injections) and in the group (group 5) treated with NDMA plus BLA 13 of 15 mice had developed lung tumors. As shown in Table 1, the posttreatment of BLA resulted in significant($P < 0.05$, Student's t-test)increase in the average number of lung tumors per mouse in NDMA-treated mice. The incidence of lung tumor-bearing mice in the group 5 tended to increase more than in the group 2, although the incidence of lung tumor-bearing mice in group 5 was not significantly higher (evaluated by qui-

Table 1. Promoting effect of basic lead acetate on the induction of mouse lung tumors by N-nitroso-dimethylamine

Group no	Treatment	Number of effective mice	Number of tumor-bearing mice	Total nodules /total mice	Nodules /mouse
1	Control	15	0	0/15	0.0
2	NDMA(three inj)	14	11	48/14	3.4
3	NDMA(two inj)	15	3	5/15	0.33
4	BLA(eighteen inj)	18	0	0/18	0.0
5	NDMA(three inj) + BLA	15	13	86/15	5.7*
6	NDMA(two inj) + BLA	18	8	14/18	0.78**

* Significantly different from group 2 at P 0.05.
** Significantly different from group 3 at P 0.01.

square test). In the group (group 3) treated with NDMA (two i.p. injections) 3 of 15 mice had developed lung tumors and 8 of 18 mice had developed lung tumors in the group (group 6) treated with NDMA (two i.p. injections) plus BLA. The incidence of tumor-bearing mice in group 6 tended to increase but was not significantly higher than that in group 3. However, the average number of lung tumors per mouse was significantly ($P < 0.01$) higher in the group 6 than in the group 3. No tumor was found in liver and kidney of any of the groups under our experimental conditions. The continuous feeding or drinking of NDMA has been reported to produce tumors in kidneys, livers and lungs of strain dd mice (Takayama and Oota 1963 and 1965; Otsuka and Kuwahara 1971; Kuwahara et al. 1972).

Our previous report (Yamane et al. 1981) showed that a high incidence of lung tumors in strain dd mice which received a total of three i.p. injections of NDMA once a week was found 6-8 months after the final injection. In this report no tumor was found in the lungs of strain ddy mice treated with BLA alone under our experimental conditions. The incidence of spontaneous lung tumor prodution in strain dd mice has been reported to be 12%

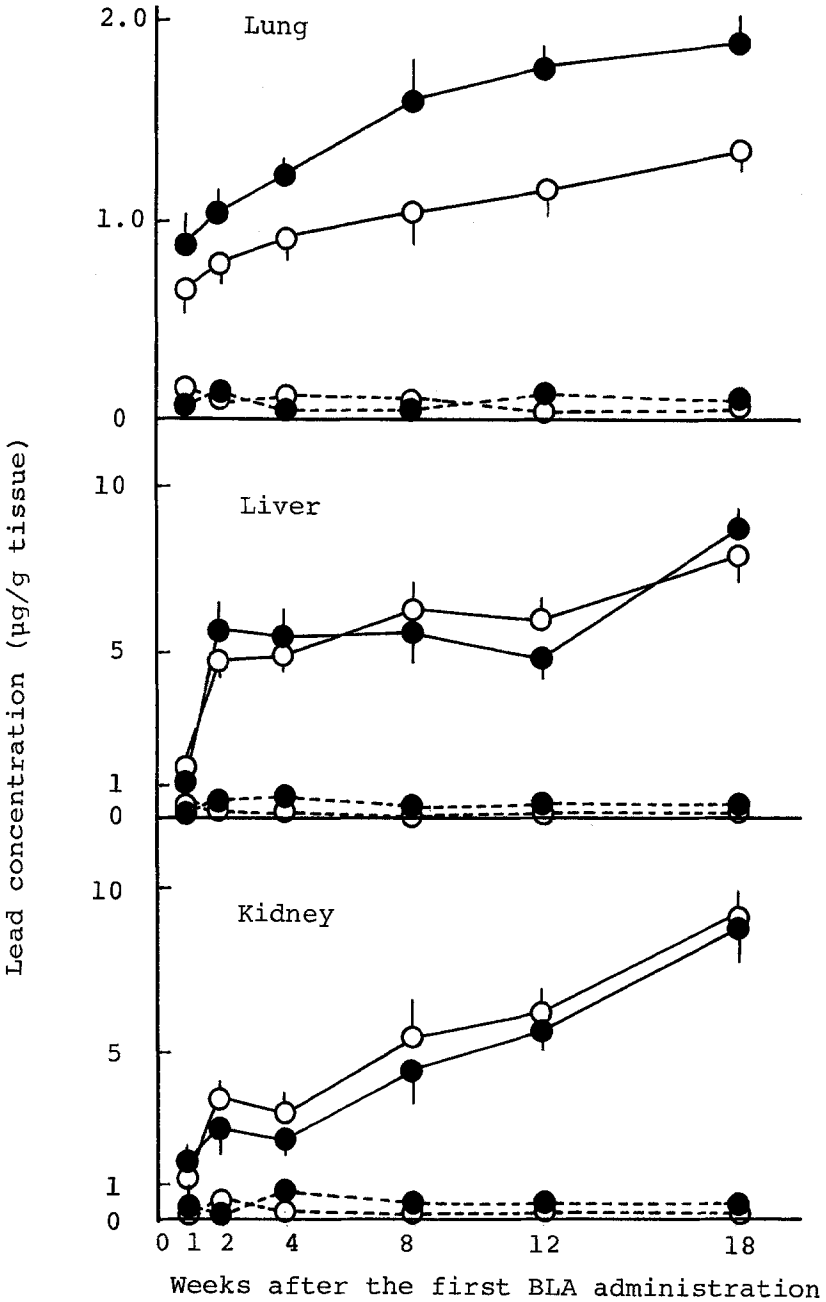

Figure 1. Lead concentrations in lung, liver and kidney of mice treated with NDMA and/or BLA. Each point is shown as mean + SD of 6-8 mice. o---o: Control group (group 1); ●---●: NDMA group (group 3); o—o: BLA group (group 4); ●—●: NDMA + BLA group (group 6).

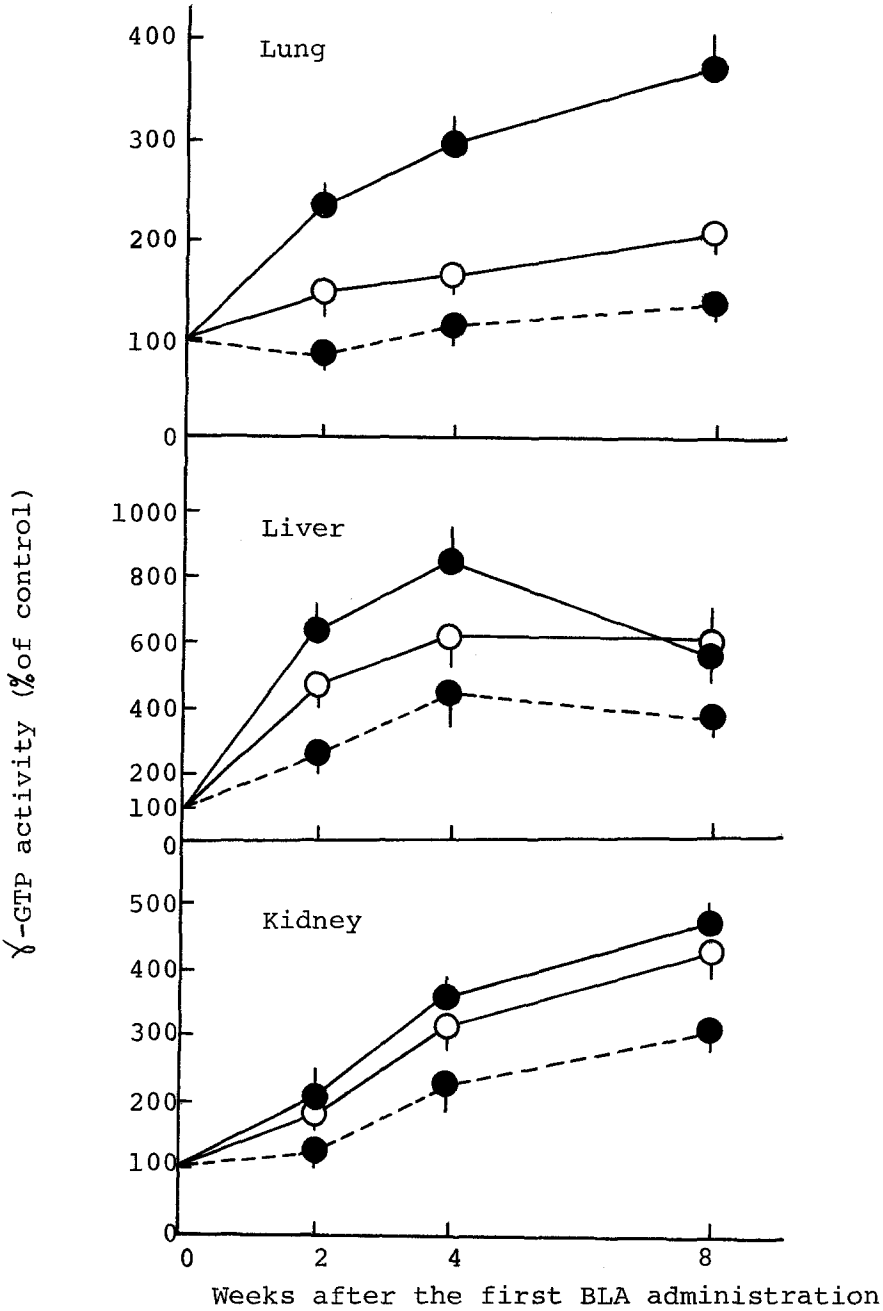

Figure 2. Effect of BLA administration on γ-GTP activity in the lung, liver and kidney microsomes of NDMA-treated mice. Each point is shown as mean + SD of 6-8 mice.
●---●: NDMA group (group 3); o—o: BLA group (group 4);
●—●: NDMA + BLA (group 6).

(Shimkin 1955) or below 12%(Mori 1961) from the 12 month after the birth until the 18 month. In our previous report lung tumors observed in NDMA-treated mice were classified into adenoma and adenocarcinoma. In this report the number of tumors produced was compared and classification of adenoma and adenocarcinoma was not taken into consideration. On the other hand, not only the carcinogenicity of lead compounds but also their cocarcinogenicity as an initiator or promoter has been studied. It has been reported that the p.o. administration of BLA or lead acetate to rats mainly produces tumors in the kidneys (Boyland et al. 1962; Van Esch et al. 1962; Ito et al. 1971) and the s.c. injections of lead phosphate to rats also produces tumors in the kidney (Zollinger 1953; Balo et al. 1965). In Swiss mice the dietary BLA has been reported to produce mainly tumors in the kidneys (Van Esch and Kroes 1969). The carcinogenicity of BLA in lungs of mice have been examined in strain A mice with high incidence of spontaneous lung tumor production (Stoner et al. 1976; Poirier et al. 1984). However, there are only a few reports on the cocarcinogenicity of lead in vivo. Shakerin et al. (1965) reported that in the groups fed a diet containing BLA plus 2-acetylaminofluorene a greater incidence of carcinoma was found in the liver and kidney, as compared with groups fed 2-acetylaminofluorene or BLA alone. Hiasa et al. (1983) reported a promoting effect of BLA on the development of kidney tumors in rats exposed to N-ethyl-hydroxyethylnitrosamine. On the other hand, Kobayashi and Okamoto (1974) reported that lead oxide showed a cocarcinogenic effect on the production of hamster lung tumors by benzo(a)pyrene. In this report no tumor was found in the livers and kidneys of mice treated with NDMA and/or BLA under the experimental conditions tested.

The concentration of lead in the lung was measured at 1, 2, 4, 8, 12 and 18 weeks after the start of lead administration. Mice were sacrificed immediately before the next scheduled injection of BLA. The lead concentration in the lung of the groups treated with BLA alone and with NDMA was considerably high and the concentration of lead in the group 6 (NDMA plus BLA) was always higher than that in the group 4 (BLA alone), as shown in Figure 1.

It has been reported that γ-GTP activity increases in neoplastic and prenoeplastic liver cells of mice and rats (fiala et al. 1976; Jalanko and Ruoslahti 1979). In this paper γ-GTP activity of the lung, liver and kidney was determined according to the method of Jalanko and Ruoslahti. The activity of liver and kidney tended to be increased by the administration of NDMA or BLA and increased little more by the administration of NDMA plus BLA than by that of NDMA alone or BLA alone. In contrast to liver and kidney, the γ-GTP activity of lung

was increased much more by the administration of NDMA plus BLA than by NDMA alone or BLA alone, as shown in Figure 2. This result may indicate that the administration of BLA had a more significant effect on the tumorigenesis of lung than of liver or kidney under our experimental conditions.

There are no conclusive epidemiological studies indicating that lead is a human carcinogen but studies showning a slight (although not significant) excess of deaths due to the digestive and respiratory cancers among lead smelters workers. In animal expeiments lead oxide has been reported to show a cocarcinogenic effect on the induction of hamster lung tumors by benzo(a)pyrene (Ko bayashi and Okamoto 1974). Therefore more attention should be placed to lead compounds as a cocarcinogen or promoting factor of environmental chemical carcinogenesis.

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