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METABOLISM IN VIVO OF DIOXANE: EFFECT OF INDUCERS AND INHIBITORS OF HEPATIC MIXED-FUNCTION OXIDASES*

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In the preceding communication (1), we summarized our studies on the metabolism in vivo of dioxane which identified p-dioxane-2-one to be the major urinary metabolite. It is well documented that the activity of mixed-function oxidases (MFO) metabolizing foreign compounds can be modulated by various inducers, repressors and inhibitors (2-4). In order to investigate the involvement of MFO in the metabolism in vivo of dioxane, the effect of several such agents on the excretion of p-dioxane-2-one was studied.

Male Sprague-Dawley rats (95-130 g) were used throughout. Three typical inducers of MFO were employed: phenobarbital (PB), the polychlorinated biphenyls, Aroclor 1254 (PCB) (Monsanto Chemical Co.), and 3-methylcholanthrene (MC). PB was dissolved in 0.9% saline and administered i.p. at a dose of 80 mg/kg daily for 4 consecutive days prior to dioxane administration. PCB and MC were dissolved in corn oil; PCB was given at a single dose of 500 mg/kg 4 days prior to dioxane administration and MC at 40 mg/kg 24 hr prior to dioxane administration. Control rats received equivalent amounts of saline or corn oil. To repress the synthesis of cytochrome P-450 (5), cobaltous chloride (60 mg/kg) was injected s.c. 24 hr prior to dioxane administration. Dioxane (3 g/kg) was given i.p. and urine samples were collected at 8- or 12-hr intervals and treated as previously (1). 2,4-Dichloro-6-phenylphenoxy ethylamine (DPEA), a potent, long-acting inhibitor of MFO (6, 7) (gift of Dr. R. E. McMahon, Eli Lilly Co.), was dissolved in saline and given at the dose of 15.9 mg/kg 30 min prior to dioxane administration and 8, 16 and 24 hr thereafter. The amount of p-dioxane-2-one present in urine was determined by analytical gas chromatography using purified synthetic reference compound as standard.

Figure 1 shows the effect of PB pretreatment on the excretion of p-dioxane and p-dioxane-2-one. PB significantly increased the total amount of the metabolite excreted (227 mg/200 g body weight for control rats and 370 mg for PB-treated rats). In addition, the time required for peak excretion was reduced from between 20 to 28 hr for control rats to about 12 hr for PB-treated rats. Administration of DPEA to PB-treated rats partially blocked the stimulatory effect of PB in the first 16 hr; DPEA alone was also inhibitory.

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Similar results were obtained when rats were pretreated with PCB. While there was virtually no effect with MC, cobaltous chloride decreased metabolite excretion.

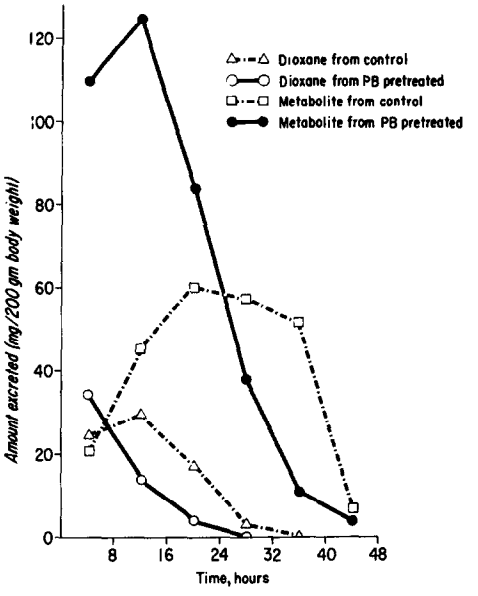

Fig. 1. Effect of phenobarbital on the excretion of dioxane and p-dioxane-2-one.

To ensure that the effects observed above were not due to the modulation of renal excretory function by various agents, the results were also expressed on the basis of mg creatinine excreted. Creatinine was determined by the modified micromethod of Folin's creatinine assay (8). Administration of dioxane caused a decrease in creatinine excretion in the first 8 hr. Pretreatment with the various inducers had no clear-cut effect on the creatinine excretion; however, cobaltous chloride substantially lowered creatinine excretion. Table 1 shows the effect of PB administration on the metabolite excretion, expressed on the basis of mg creatinine excreted. Clearly, the stimulatory effect of PB was independent of renal excretory function. DPEA substantially curtailed the PB effect in the first 16 hr. Similarly, the stimulatory effect of PCB was independent of renal excretory function and was curtailed by DPEA.

TABLE 1. Effect of PB and of PB + DPEA on p-dioxane-2-one excretion*

Treatment	Time period					
	0-8 hr	8-16 hr	16-24 hr	24-32 hr	32-40 hr	40-48 hr
Control	10.7	15.9	18.0	21.1	21.0	2.2
PB	52.4	45.4	22.4	15.9	3.6	1.1
PB + DPEA	27.6	35.0	19.0	20.5	21.8	3.0

*Values are expressed as mg p-dioxane-2-one/mg of creatinine. DPEA was given 0.5 hr before and 8, 16 and 24 hr after the administration of dioxane.

To confirm the above findings, experiments were carried out using uniformly labeled [^{14}C]-dioxane. Rats were given [^{14}C]-dioxane along with unlabeled carrier and urine samples were collected for 12-hr periods. The urine obtained was alkalinized to pH > 12 with 10 per cent NaOH, a treatment that converts p-dioxane-2-one into 2-hydroxyethoxyacetate (1). The alkalinized urine was then separated on anion-exchange columns (Bio-Rad AG 1 x 8, acetate form, mesh 200-400, 0.7 x 4 cm). Two fractions were collected: a non-anionic fraction (eluted by distilled water) and an anionic fraction (eluted by 0.5 M NaCl). The former fraction was mainly composed of p-dioxane while the latter contained mainly 2-hydroxyethoxyacetate anion. Results obtained from calculations of the radioactivity present in these fractions indicated close agreement with those obtained by analytical gas chromatography. Pretreatment with either PB or PCB increased the total excretion of ^{14}C-labeled metabolite and reduced the time required for peak excretion.

The results strongly suggest the involvement of typical microsomal MFO in the metabolism *in vivo* of dioxane. Of the three typical inducers of MFO used, PB and PCB significantly increased the amount of metabolite excreted and shortened the peak excretion time. Although MC had virtually no effect, it is now well established that PB and MC represent two distinct classes of agents (2-4) which induce cytochromes P-450 and P-448 respectively. The PCB, on the other hand, appear to have a wide spectrum of inducing activity, inducing both types of cytochromes (9, 10). The results suggest the involvement of P-450 types of cytochromes in the metabolism *in vivo* of dioxane. The observations that DPEA, a long-acting inhibitor of MFO, inhibits the excretion of p-dioxane-2-one from untreated and PB- and PCB-treated rats, and that cobaltous chloride, a repressor of cytochrome P-450 synthesis, also inhibits the metabolite excretion lend further support to the involvement of MFO.

The relationship between the metabolism *in vivo* of dioxane and its acute toxicity and/or carcinogenicity is a subject of great interest in view of the possibility that p-dioxane-2-one may be carcinogenic. Preliminary toxicity data of p-dioxane-2-one indicate that the lactone (LD_{50} = 0.75 g/kg) is considerably more toxic than dioxane. Agents that modify the metabolism of dioxane may therefore be expected to modify its toxicity; this is in fact the case for certain inducers and inhibitors of MFO. Pretreatment with PCB, which increases the metabolism of dioxane, significantly increases its toxicity (LD_{50} = 5.3 ± 0.1 g/kg for control and 4.4 ± 0.1 g/kg for PCB-treated rats). Simultaneous administration of DPEA (which inhibits the metabolism) appeared to be protective; at a dioxane dose of 4.55 g/kg, the mortality rate was decreased from 70 per cent for PCB-treated rats to 30 per cent for rats treated with PCB + DPEA. Pretreatment with MC, which had virtually no effect on the excretion of the metabolite, had no significant effect on the LD_{50}. However, PB seems to be an exceptional case since PB-pretreatment appeared to have no significant effect on the LD_{50} of dioxane in spite of increasing its metabolism. The reason for this difference is not known; one possibility is that the lactone may be further metabolized to exert its toxic effect. In this regard, preliminary studies indicate that PCB-pretreatment increases the acute toxicity of the lactone; at a lactone dose of 0.7 g/kg, the mortality rate was increased from 40 per cent to 90 per cent after

pretreatment with PCB. Experiments are currently in progress to elucidate the role of the metabolism in the toxicity and carcinogenicity of dioxane.

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REFERENCES

1. Y.-T. Woo, J. C. Arcos, M. F. Argus, G. W. Griffin and K. Nishiyama. Biochem. Pharmac. 26, 1535 (1977).
2. A. H. Conney. Pharmac. Rev. 19, 317 (1967).
3. R. Kuntzman. Ann. Rev. Pharmac. 9, 21 (1969).
4. J. R. Gillette, D. C. Davis and H. A. Sasame. Ann. Rev. Pharmac. 12, 57 (1972).
5. T. R. Tephly and P. Hibbeln. Biochem. biophys. Res. Commun. 42, 589 (1971).
6. C. J. Parli, N. Wang and R. E. McMahon. J. Pharmac. exp. Ther. 180, 791 (1972).
7. C. J. Parli, N. W. Lee and R. E. McMahon. Drug Metab. Dispos. 1, 628 (1973).
8. P. B. Hawk, B. L. Oser and W. H. Summerson. Practical Physiological Chemistry, 13th Edn., p. 899. McGraw-Hill, New York (1954).
9. A. P. Alvares, D. R. Bickers and A. Kappas. Proc. natn. Acad. Sci. U.S.A. 70, 1321 (1973).
10. A. P. Alvares and A. Kappas. FEBS Letters 50, 172 (1975).