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SHORT COMMUNICATION

Inhibition of Methanol and Ethanol Oxidation by Pyrazole in the Rat and Monkey *in Vivo*

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SUMMARY

Pyrazole has no effect on the catalase-peroxidative system but exerts a pronounced inhibition of liver alcohol dehydrogenase and ethanol oxidation *in vivo*. Methanol oxidation is decreased by pyrazole in the rat, where a role of liver alcohol dehydrogenase has not previously been established. However, the inhibitory effect of pyrazole on methanol oxidation is more marked in the monkey, where alcohol dehydrogenase is the major catalyst for methanol oxidation.

Methanol poisoning depends upon its metabolism to toxic products, which are responsible for a profound acidosis and blindness and which eventually may lead to death (1). Two enzymatic systems have been examined for their role in the metabolism of methanol: liver alcohol dehydrogenase and the catalase-peroxidative system involving hepatic catalase and a source of peroxide generation. Employing the catalase inhibitor 3-amino-1,2,4-triazole and competitive substrates for both liver alcohol dehydrogenase and catalase-H_2O_2 (complex I) *in vitro* and *in vivo*, Tephly *et al.* (2) showed that in the rat the catalase-peroxidative system plays a major role in the metabolism of methanol. Unlike the effects seen in the rat, AT[1] does not inhibit methanol oxidation in the monkey, and experiments using alternative substrates for the systems revealed a pattern consistent with a predominant role for alcohol dehydrogenase in methanol oxidation (3). The lack of an effective peroxidative mechanism for methanol oxidation in the monkey may be explained by recent studies, which indicate that the peroxidative capacity of catalase and the activity of certain peroxide-generating enzymes in hepatic peroxisomes are decreased in the monkey (4, 5).

Theorell and Yonetani (6) have shown that pyrazole is a potent inhibitor of liver alcohol dehydrogenase and that it competes with ethanol for the ethanol-binding site. Pyrazole, therefore, may provide a direct test for the role of alcohol dehydrogenase in the metabolism of methanol in much the same way that AT does for the catalase-peroxidative system. A number of investigators (7–10) have recently shown that pyrazole and certain of its derivatives inhibit ethanol oxidation in the rat *in vivo*. Evidence is presented in this report which shows that pyrazole does not affect peroxidative methanol oxidation and that it is

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[1] The abbreviation used is: AT, 3-amino-1,2,4-triazole.

an effective substance for studying the role of alcohol dehydrogenase in the metabolism of methanol *in vitro* and *in vivo*.

Isotopes were purchased from New England Nuclear Corporation and were diluted with inert alcohol and water to a specific activity of 25,000 dpm/ml. Pyrazole was purchased from K and K Laboratories. Anisole, dimethoxyethane, methanol, ethanol, and 2,4-dioxane were purchased from Mallinckrodt. Cab-O-Sil, 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP), and 2,5-diphenyloxazole (PPO) were purchased from Packard Instrument Company. 3-Amino-1,2,4-triazole was purchased from Eastman Organic Chemicals.

Studies on the peroxidative oxidation of methanol and on the inhibition of catalase activity by AT and pyrazole were performed as described previously (8, 9).

Male Sprague-Dawley rats (250–350 g) and male rhesus monkeys (3 kg) were employed in experiments *in vivo*. All animals received commercially available diets and water ad libitum up to initiation of experimentation. Each animal was administered intraperitoneal injections of ethanol-1-^{14}C (21.7 mmoles/kg) or methanol-^{14}C (32.3 mmoles/kg) solutions (1 g/kg, 20% alcohol in 0.9% NaCl solution), after which time it was immediately placed in a glass metabolism chamber (2, 3). Aliquots (1 ml) of the sodium hydroxide solutions containing expired $^{14}CO_2$ were removed at specified time intervals and added to 15 ml of a scintillation fluid composed of 2,4-dioxane (75%, v/v), dimethoxyethane (5%, v/v), anisole (12.5%, v/v), 2,5-diphenyloxazole (1.2%, w/v), 1,4-bis[2-(5-phenyloxazolyl)] benzene (0.05%, w/v), and Cab-O-Sil (5%, w/v). The radioactivity of the samples was determined with a Packard Tri-Carb liquid scintillation spectrometer. The efficiency of this system was about 70%.

The fact that the catalase-peroxidative system coexists with the liver alcohol dehydrogenase system *in vivo* necessitated the demonstration of the specificity of pyrazole for the latter system. The effect of pyrazole on catalase was examined in three ways in these studies.

It can be seen in Fig. 1 that pyrazole has no effect on catalase activity in the presence and absence of a peroxide-generating system composed of glucose oxidase and glucose. 3-Amino-1,2,4-triazole exerts the expected inhibition of catalase activity when a source of peroxide is supplied (Fig. 1). It has been shown that substrates for catalase-H_2O_2 (complex I) protect catalase from inhibition by AT *in vitro* and *in vivo* (12). Pyrazole has no effect on the inhibition of catalase activity produced by AT (Fig. 1). Therefore, pyrazole appears not to be a substrate for catalase-H_2O_2 (complex I).

AT produces profound inhibition of hepatic catalase activity *in vivo* (13). However, pyrazole has no effect on the activity of hepatic catalase *in vivo* (Table 1). Furthermore, pyrazole has no effect on the peroxidative oxidation of methanol in a purified system containing crystalline beef liver catalase, glucose, and glucose oxidase (Table 2). Table 2 also shows the expected inhibition of methanol oxidation by AT (15) and the lack of reversal of this inhibition by pyrazole that would be observed by substrates for catalase-H_2O_2 (complex I).

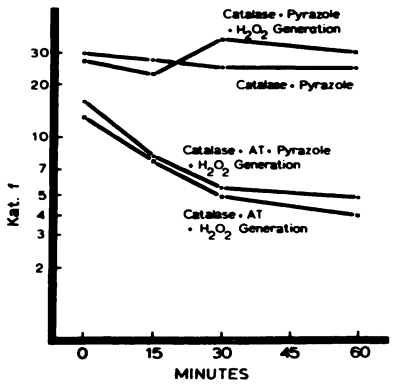

FIG. 1. *Effect of pyrazole on crystalline catalase activity*

Samples were withdrawn at indicated time intervals and evaluated for catalase activity by the method of Feinstein (11). The following concentrations were employed where indicated: pyrazole, 10^{-2} M; AT, 3×10^{-2} M; glucose, 40 mg/ml; and glucose oxidase, 0.4 mg/ml, in a final volume of 5 ml. Crystalline beef liver catalase (30 kat.*f*) was added at zero time, and activity represents that remaining after incubation at 37° in Na_2HPO_4–KH_2PO_4 buffer, 0.1 M, pH 7.4.

TABLE 1

Effect of pyrazole and aminotriazole on rat hepatic catalase activity in vivo

Rats were given pyrazole (2.94 mmoles/kg), AT (11.9 mmoles/kg), or 0.9% NaCl intraperitoneally. Livers were removed 1 hr after AT and 15 min after pyrazole treatment, and catalatic catalase activity was determined by the method of Feinstein (11) as described previously (8). Values represent the mean of three experiments ± standard error.

Treatment	Catalase activity
	kat.f/g liver
None	517 ± 44
AT	28 ± 5
Pyrazole	525 ± 72
AT + pyrazole	37 ± 3

TABLE 2

Effect of pyrazole on peroxidative oxidation of methanol in vitro

Peroxidative catalase activity was measured as described previously (5). The concentrations of pyrazole and AT were 0.02 M and 0.1 M, respectively. Reaction mixtures contained crystalline beef liver catalase (1000 units, Sigma Chemical Company), glucose (4 mg/ml), glucose oxidase (0.2 mg/ml, Signal Chemical Company), methanol (0.1 M), and Na_2HPO_4–KH_2PO_4 buffer (0.1 M, pH 6.8) in a final volume of 5 ml. Incubations were carried out at 37°, and reactions were started by the addition of methanol after 15 min of incubation. Formaldehyde content was determined by the method of Nash (14) 30 min after the addition of methanol.

Treatment	Methanol oxidation
	mμmoles/HCHO formed/ml/min
None	16.5
Pyrazole	16.5
AT	0.5
Pyrazole + AT	0.0

Theorell and his colleagues have shown that pyrazole competes with ethanol for the alcohol-binding site on liver alcohol dehydrogenase and that pyrazole was a very potent inhibitor of this enzyme (6, 16). Recently, it has been shown (7, 9, 10) that pyrazole and certain derivatives inhibit ethanol metabolism *in vivo* in the rat. These studies have been confirmed in this laboratory (8). At a dose of 2.94 mmoles/kg of pyrazole, 80% inhibition of ethanol oxidation was observed *in vivo* (Fig. 2). These results are in accord with the view that ethanol is metabolized in the rat by liver alcohol dehydrogenase.

Figure 3 shows that pyrazole at a dose of 2.94 mmoles/kg inhibits the oxidation of methanol in the rat, but to a lesser degree than the oxidation of ethanol. This was expected, since Tephly *et al.* (2) have shown that in the rat methanol is oxidized to a large part by a catalase-peroxidative route. However, these data indicate that liver alcohol dehydrogenase also plays a role in the oxidation of methanol in the rat *in vivo*.

Previous studies by Makar *et al.* (3) indicated that whereas the peroxidative oxidation of methanol in the rat is a major pathway, its role in the monkey is minimal. The current report further substantiates the role of liver alcohol dehydrogenase as the primary catalyst for methanol oxidation in the monkey. Figure 4 shows the effect of 2.94 mmoles/kg of pyrazole on the oxidation of methanol in the monkey *in vivo*. At a dose of 32.3 mmoles/kg of methanol, 80% inhibition was seen during the period of measurement.

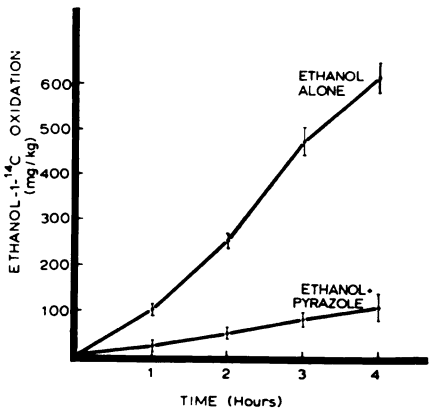

FIG. 2. *Effect of pyrazole on ethanol oxidation in the rat in vivo*

1-^{14}C-Ethanol (21.7 mmoles/kg) was injected intraperitoneally. When administered, pyrazole (2.94 mmoles/kg) was injected intraperitoneally 15 min before 1-^{14}C-ethanol. Each point represents the mean value of four animals ± standard error.

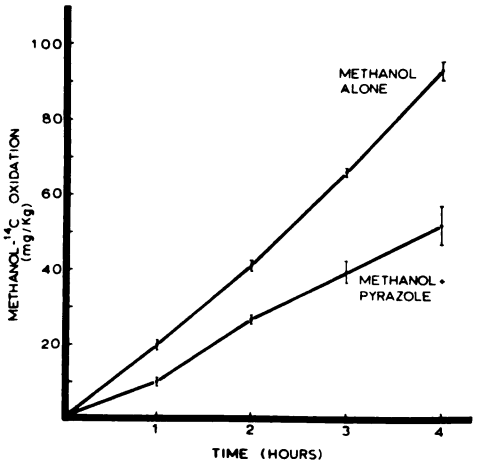

FIG. 3. *Effect of pyrazole on methanol oxidation in the rat in vivo*

^{14}C-Methanol (32.3 mmoles/kg) was injected intraperitoneally. When administered, pyrazole (2.94 mmoles/kg) was injected intraperitoneally 15 min before ^{14}C-methanol. Each point represents the mean value of four animals ± standard error.

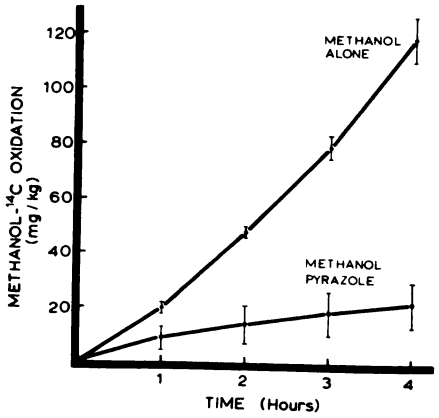

FIG. 4. *Effect of pyrazole on methanol oxidation in the monkey in vivo*

^{14}C-Methanol (32.3 mmoles/kg) was injected intraperitoneally. When administered, pyrazole (2.94 mmoles/kg) was injected intraperitoneally 15 min before ^{14}C-methanol. Each point represents the mean value of three animals ± standard error.

After treatment with methanol and pyrazole as described in Fig. 4, monkeys exhibited severe toxicity, as evidenced by weight loss, lethargy, pallor, and death in about 3–4 days. Therefore, a lower dose of pyrazole was used in order to find a dose that might prove useful in inhibiting methanol oxidation without producing toxic effects in the monkey. Figure 5 shows the results obtained from two monkeys which received methanol (32.3 mmoles/kg) followed 4 hr later by pyrazole (0.74 mmole/kg) and 8 hr later by ethanol (5.4 mmoles/kg). The average rate of methanol oxidation in these animals was 22 mg/kg/hr, which was reduced to 11 mg/kg/hr by the injection of pyrazole. The rate for the first hour after ethanol was about 5 mg/kg/hr, which returned over the next 3 hr to that seen with pyrazole alone. Studies performed with monkeys receiving only methanol (32.3 mmoles/kg) showed that the rate of oxidation of this dose of methanol remains linear throughout the 12-hr period of experimentation illustrated in Fig. 5. Table 3 shows that in two monkeys receiving 5.43 mmoles/kg of ethanol 4 hr after 32.3 mmoles/kg of methanol, inhibition of about 50% was observed during the first hour after ethanol. This inhibition was slightly less than that observed in a previous study (3) when the alcohols were administered together. This may be explained by the difference in experimental design or by

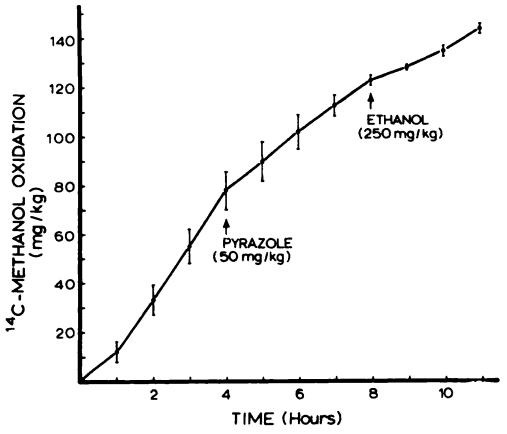

FIG. 5. *Effect of pyrazole and ethanol on methanol oxidation in the monkey in vivo*

^{14}C-Methanol (32.3 mmoles/kg) was injected intraperitoneally. Pyrazole (0.735 mmole/kg) and ethanol (5.43 mmoles/kg) were injected intraperitoneally at 4 and 8 hr, respectively, following the initial ^{14}C-methanol administration. Each point represents the mean value of two animals with the corresponding range at each time interval.

TABLE 3

Effect of ethanol on methanol-^{14}C oxidation in the monkey

The rate of methanol oxidation represents the rate over the first hour following the administration of 250 mg (5.43 mmoles)/kg of ethanol. Ethanol was injected intraperitoneally 4 hr after the injection of 32.3 mmoles/kg of methanol.

Experiment	Methanol oxidation	
	Before ethanol	After ethanol
	mg/kg/hr	*mg/kg/hr*
1	28	17
2	27	12

differences in the animals. The animals treated as described in Fig. 5 did not display any observable toxicity. They gained weight and showed, up to at least 1 month later, none of the toxic manifestations seen with the 2.94 mmoles/kg dose of pyrazole. Further studies are required before the latent toxicity of pyrazole can be explained, but this toxicity does indicate that more work must be done before pyrazole is used in man.

These studies show that pyrazole does not react with the catalase-peroxidative system and is useful for studying the participation of liver alcohol dehydrogenase in the metabolism of a substrate such as methanol *in vivo*. In this respect pyrazole compares favorably with 3-amino-1,2,4-triazole, which has been useful for studying the role of the catalase-peroxidative system in the oxidation of methanol *in vivo* (2, 3). It has been shown by Tephly *et al.* (2) that the catalase-peroxidative system plays a prominent part in the oxidation of methanol in the rat. The present investigation shows that liver alcohol dehydrogenase also plays a substantial role in the metabolism of methanol in this species. In the monkey, where aminotriazole does not inhibit methanol metabolism *in vivo* (3), pyrazole profoundly inhibited methanol metabolism. This is in agreement with the studies of Makar *et al.* (3), which indicated that alcohol dehydrogenase is the main catalyst in methanol oxidation in this species. Pyrazole is not a broad inhibitor of dehydrogenases (17), and it does not inhibit hepatic aldehyde dehydrogenase.[2] It has recently been reported that pyrazole is capable of inhibiting catalase activity *in vivo* (18). At a dose of 2.94 mmoles/kg, pyrazole in the presence of methanol did not inhibit hepatic catalase activity *in vivo*.

Pyrazole produced no observable toxicity in rats at the doses employed in this report. In the monkey, pyrazole at doses of 2.94 mmoles/kg produced a latent toxicity, which would exclude its use at this dose in the treatment of methanol poisoning in humans. However, at the lower dose of 0.74 mmole/kg, no toxicity was observed for the monkey although pyrazole effected 50% inhibition of methanol oxidation, which was prolonged and is consistent with observations made in the rat. Lester *et al.* (7) have reported that in the rat ethanol oxidation rates return to normal about 144 hr after a dose of pyrazole of 2.2 mmoles/kg. These studies have been confirmed by this laboratory. Low doses of ethanol have been shown to inhibit methanol oxidation in the monkey (3), but the effect lasts only for several hours because ethanol is rapidly metabolized (150 mg/kg/hr). In the present investigation, treatment with ethanol (5.4 mmoles/kg) following pyrazole depressed methanol oxidation to about 20% of the control value during the first hour after administration. It is possible that with lower doses of pyrazole or one of its more potent derivatives (3, 16), in combination with a low dose of ethanol, effective control of methanol oxidation can be achieved without superimposing the toxicity of either or both of these substances on the toxicity of methanol.

Lelbach (19) has recently observed that prolonged administration of ethanol together with pyrazole produced hepatic lesions and death in rats. Under the conditions of the experiments reported in this paper, liver pathology was evaluated by light microscopy and by measurement of serum glutamate-oxalacetate transaminase levels. No statistically significant increases in the levels of this enzyme were observed.

[2] Unpublished observations.

Light microscopic assessment of the hepatic histology of rats and monkeys revealed no abnormalities up to 72 hr after treatment. Furthermore, high doses of pyrazole (1 g/kg) were also employed in rats, and no evidence of hepatic pathology was found, although profound hypoglycemia was observed at this high dose. No hypoglycemia was observed in monkeys up to 72 hr after the administration of pyrazole and methanol in the current studies. However, the use of pyrazole in man should be viewed as hazardous at this time.

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