Short Communication

Metabolism of dacarbazine by rat liver microsomes: contribution of CYP1A enzymes to dacarbazine N-demethylation

(Received July 22, 1997; accepted December 3, 1997)

This paper is available online at http://www.dmd.org

ABSTRACT:
The N-demethylation of dacarbazine in liver microsomes was significantly increased by treatment of rats with β-naphthoflavone, dexamethasone, or phenobarbital. However, the extent of increase in the N-demethylation observed in β-naphthoflavone-treated rats was much greater than that observed in dexamethasone-treated rats. A good correlation between N-demethylation of dacarbazine and O-deethylation of phenacetin was observed when a low concentration of phenacetin was used. Furthermore, the activity of dacarbazine N-demethylase in rat liver microsomes was highly correlated with the amounts of CYP protein immunochromically determined with anti-rat CYP1A2 antibodies. In addition, antibodies to rat CYP1A2, and furafylline and α-naphthoflavone, which are known inhibitors of CYP1A enzymes, exhibited inhibitory effects on dacarbazine N-demethylation. These results indicated that CYP1A enzymes may be responsible for N-demethylation of dacarbazine in rat liver microsomes.

5-(3,3-Dimethyl-1-triazeno) imidazole-4-carboxamide (dacarbazine) is an antineoplastic drug which is classified as an alkylating agent (Newell et al., 1987). It is used extensively as a single drug in the treatment of metastatic malignant melanoma and in combination with other drugs for treating renal adenocarcinoma, soft tissue sarcoma, solid tumor, and malignant lymphomas (Lee et al., 1987; Beal et al., 1987; Skibba and Bryan, 1970; Hill, 1975). Furthermore, it has been suggested that both 5-(3-hydroxymethyl-3-methyltriazen-1-yl)-imidazole-4-carboxamide and 5-(3-methyltriazen-2-yl) imidazole-4-carboxamide were dacarbazine metabolites in mice, rats, and humans, and that 5-(3-hydroxymethyl-3-methyltriazen-2-yl) imidazole-4-carboxamide decomposes spontaneously to form aminimidazole carboxamide, with concomitant alkylation of cellular DNA (1, 10–12Newell et al., 1987; Beal et al., 1975; Kolar et al., 1980; Nagasawa et al., 1974; Mizuno et al., 1976). Therefore, N-demethylation of dacarbazine has been considered to be an important metabolic pathway for both the antineoplastic and carcinogenic activities of dacarbazine (Beal et al., 1975; Spassova and Golovinsky, 1985). Since several lines of evidence have demonstrated that liver microsomal enzyme(s) is responsible for N-demethylation of dacarbazine leading to the formation of monomethyl triazene imidazole carboxamide and that the P450 is responsible for the bioactivation of dacarbazine (Hill, 1975; Mudipalli et al., 1995), it is likely that the antineoplastic activity of dacarbazine may be affected by the activity of P450 responsible for N-demethylation of the drug. However, the form(s) of P450 that contribute to N-demethylation of dacarbazine are still undetermined. Therefore, this study was conducted to get better understanding of the P450 enzyme responsible for N-demethylation of dacarbazine in rat liver microsomes.

Materials and Methods

Materials. Dacarbazine was a generous gift from Kyowa Hakko KK. (Tokyo, Japan). CYP1A2 and CYP2B1/2 were purified from liver microsomes of rats treated with 3-methylcholanthrene and phenobarbital by the methods reported previously, respectively (Tamataki et al., 1983; Kitada et al., 1984). CYP3A4 and CYP2C9 were purified from human liver microsomes according to methods described elsewhere (Shimada et al., 1986; Komori et al., 1988). The antibodies for purified P450 raised to rabbit were carried out according to the method previously reported (Kamataki et al., 1976). NADP, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were obtained from Oriental Yeast (Tokyo, Japan). Other chemicals used were of the highest grade commercially available.

Animals. Male Sprague-Dawley rats (8 weeks old), obtained from Takasugi Experimental Animals Co. Ltd. (Saitama, Japan), were used throughout this study. Rats were pretreated intraperitoneally with phenobarbital-Na at doses of 80 mg/kg for 5 days, dexamethasone 50 mg/kg for 3 days, and β-naphthoflavone 40 mg/kg for 3 days. The agents for pretreatment were dissolved in corn oil except for phenobarbital which was dissolved in water. Ethanol was given orally in a solution at a 10% concentration. Rats were given free access to food and water and were killed 24 hr after the last injection. Liver microsomes were prepared by differential centrifugation as described elsewhere (20).

Assays. A typical reaction mixture consisted of 100 mM potassium phosphate buffer (pH 7.4), NADPH-generating system, and liver microsomes.
phosphate (pH 7.4), 0.1 mM EDTA, NADPH-generating system (0.33 mM NADP, 8 mM glucose 6-phosphate, 0.1 unit of glucose 6-phosphate dehydrogenase, and 6 mM MgCl₂), microsomal protein, and 0.5 mM dacarbazine. Phenacetin was dissolved in 1% methanol at a concentration of 1 mM. The concentration of dacarbazine employed in this study was decided from blood concentration reported when clinically used (Breithaupt et al., 1982; Loo et al., 1976; Buesa and Urrechaga, 1991), and the solubility of the drug in vitro. The reaction was initiated by the addition of the NADPH-generating system which had been preincubated at 37°C for 5 min, and was carried out at 37°C for 20 min with aerobic shaking. N-Demethylase activity of dacarbazine was measured by determination of formaldehyde formed according to the method of Nash (Nash, 1953). Briefly, a yellow color developed with acetylacetone in the presence of ammonium sulfate was measured fluorometrically at 510 nm (excitation was at 410 nm) by the method of Rapoport et al. (1994) with minor modifications. O-Deethylase activities of phenacetin was measured by high performance liquid chromatography according to the method of Loft et al. (1991).

Results

N-Demethylase activities of dacarbazine in liver microsomes from rats pretreated with various inducers were studied. Although treatments of rats with phenobarbital, dexamethasone, and β-naphthoflavone resulted in the increase in N-demethylation of dacarbazine, β-naphthoflavone was much more effective in inducing the N-demethylase activity than were phenobarbital and dexamethasone. Thus, the activities of dacarbazine N-demethylase in liver microsomes from untreated, phenobarbital-, dexamethasone- and β-naphthoflavone-treated rats were 155.2 ± 19.3, 318.5 ± 24.6, 356.7 ± 36.7, and 1331.9 ± 60.5 pmol/min/mg, respectively. In contrast, pretreatment of rats with ethanol did not affect dacarbazine N-demethylation. In addition, the activity of dacarbazine N-demethylase was significantly correlated with that of phenacetin O-deethylase in liver microsomes from ethanol-, phenobarbital-, dexamethasone-, β-naphthoflavone-, and untreated rats when phenacetin was used at the concentration of 10 mM (r = 0.949, p < 0.001). In contrast, no significant correlation between dacarbazine N-demethylation and phenacetin O-deethylase was observed when the activity of phenacetin O-deethylase was measured at the concentration of 100 mM. From these results, it was suggested that CYP1A enzyme may predominantly involve in N-demethylation of dacarbazine in rat liver microsomes, although contribution of P450 enzymes other than CYP1A to the reaction cannot be excluded because N-demethylation of dacarbazine was slightly but significantly increased by pretreatment of rats with phenobarbital and dexamethasone. Fig. 1 shows the correlation of the activity of dacarbazine N-demethylation and phenacetin O-deethylase. Pre-treatment of rats with ethanol did not affect the activity of phenacetin O-deethylase. As shown in fig. 2, both furafylline and α-naphthoflavone showed inhibitory effects on N-demethylation of dacarbazine.
CYP2C enzymes, affected dacarbazine N-demethylation (fig. 2A). Furthermore, dacarbazine N-demethylation was inhibited by the pre-treatment of microsomes with anti-CYP1A2 antibodies but not by pre-treatment with anti-CYP3A4, CYP2B1/2, or CYP2C9 antibodies (fig. 2B).

Discussion

It has been shown that dacarbazine N-demethylation leading to the formation of monomethyl triazene imidazole carboxamide is an important metabolic pathway for the antineoplastic activity of dacarbazine, since the metabolite liberates a carbonium ion that is responsible for the alkylating property of dacarbazine (Hill, 1975; Spassova and Golovinsky, 1985; Mudipalli et al., 1995). However, the form(s) of P450 responsible for N-demethylation of dacarbazine in rat liver microsomes was not known. Therefore, the present study was designed to clarify the P450 enzyme contribution to proposed metabolic activation of dacarbazine in rat liver microsomes.

Although N-demethylation of dacarbazine was increased by pre-treatment of rats with phenobarbital, dexamethasone, and β-naphthoflavone, β-naphthoflavone was the most effective inducer for dacarbazine N-demethylation. Furthermore, increase in activity of N-demethylation in dexamethasone-treated rats was much less than that in the amount of CYP3A enzymes immunocohemically determined. Thus, the increase in the activities in liver microsomes from phenobarbital-, dexamethasone-, and β-naphthoflavone-treated rats were about 2-fold, 2.3-fold, and 8.5-fold, respectively. On the other hand, immunoblot analysis with anti-CYP1A2 antibodies revealed that the amount of CYP1A2 immunocohemically determined in β-naphthoflavone- and dexamethasone-pretreated rats were about 6-fold and 1.7-fold higher than that of controls, respectively. In contrast, the amount of CYP3A enzymes immunocohemically determined with anti-CYP3A4 antibodies was increased by about five times in dexamethasone-pretreated rats but not in β-naphthoflavone-pretreated rats. In addition, dacarbazine N-demethylation activity was significantly correlated with phenacetin O-deethylation measured at a low, but not at a high, concentration of phenacetin. Since O-deethylation of phenacetin by rat liver microsomes has been demonstrated to be biphasic and CYP1A2 has been shown to be a high affinity phenacetin O-deethylase in rat livers (Boobis et al., 1981; Seardic et al., 1990), the correlation between dacarbazine N-demethylation and phenacetin O-deethylation at low concentration was in accord with the results that β-naphthoflavone was the most effective inducer for dacarbazine N-demethylation in rat liver microsomes. These results were also supported by the findings that the activity of dacarbazine N-demethylation was positively correlated with the amount of CYP1A2 immunochemically determined and was inhibited by anti-CYP1A2 antibodies, whereas neither anti-CYP2B1/2 antibodies nor anti-CYP3A4 antibodies exerted any significant effects on dacarbazine N-demethylation in liver microsomes of β-naphthoflavone-treated rats. Together with these results, it was indicated that although the contribution of CYP2B and CYP3A enzymes to the reaction cannot be excluded at present, CYP1A2 may, at least, be one of the major forms of P450 responsible for dacarbazine N-demethylation in rat liver microsomes.

Since N-demethylation of dacarbazine appears to be the first metabolic pathway for bioactivation of the drug to antineoplastic species, the results presented here indicated that the activity of CYP1A1 and/or 2 may play an important role in cancer chemotherapy using dacarbazine. It has been known that the CYP1A2 isofrom is one of the constituively expressed form of human P450s (Shimada et al., 1994), and that metabolism of phenacetin in vivo is altered by cigarette smoking (Pantuch et al., 1974). Therefore, as in the case of rats, if human CYP1A1 and/or 2 isofrom was capable of metabolizing dacarbazine via N-demethylation, it is possible to assume that the level of CYP1A1 enzymes affects the therapeutic and/or toxic effects of dacarbazine in human. However, the form of P450 that contributes to