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Effects of Cigarette Smoke Condensate on the Activities of Xenobiotic Metabolizing Enzymes in Primary Cultured Rat Hepatocytes

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Abstract – The purpose of this study is to evaluate the effect of cigarette smoke condensate (CSC) on toxification/detoxification metabolic pathway in primary cultured rat hepatocytes. We measured the activities of cytochrome P450 monooxygenases (CYP450s) and UDP-glucuronyltransferase, sulfotransferase and glutathione-S-transferase in CSC-treated rat hepatocytes. CSC significantly increased the activities of hepatic CYP4501A1 and CYP4501A2 to 7.5 fold and 1.6 fold respectively, compared with control level. However, CSC did not affect the activities of conjugation enzymes. We also examined if treatment of CSC could change the cytotoxicity of acetaminophen (AA) through modulation of metabolizing enzymes. In rat hepatocytes, pretreatment with CSC potentiated the cytotoxicity of AA. This result indicates that potentiation of AA toxicity by CSC pretreatment may be related to induction of CYP4501A1 and CYP4501A2.

Keywords □ cigarette smoke condensate, metabolizing enzymes, acetaminophen

INTRODUCTION

The cigarette smoke condensate (CSC) is carcinogenic to several animal species (Wynder and Hoffmann, 1967; 1968). It has been reported that a number of tumor initiators, especially polynuclear aromatic hydrocarbons are present in tobacco smoke (Hoffmann, *et al.*, 1971). In addition bioassays using mouse skin and hamster inhalation have confirmed the presence of tumor promotors in tobacco smoke. These substances potentiate the action of tumor initiators, which would otherwise be present at concentrations below their threshold values for complete carcinogenic activity (Van Duuren, *et al.*, 1971; Hecht, *et al.*, 1975). Numerous epidemiological and animal studies have established a strong correlation of tobacco consumption with increased incidence of cancer (Blot, *et al.*, 1988). One of many plausible mechanisms, which underlie the increased risk of cancer associated with tobacco consumption, is the induction of microsomal enzymes (e.g., specific forms of cytochrome P450) that catalyze the metabolic activation of tobacco constituents or other carcinogens. In this study, we examined whether CSC could affect the metabolic pathway through the change of phase I and phase II enzymes *in vitro*.

Large doses of acetaminophen (AA) cause hepatic necrosis through the formation of an electrophile, *N*-acetyl-*p*-benzoquinoneimine (Boyd and Bereezky, 1966). This reactive intermediate is conjugated with glutathion (Mitchell, *et al.*, 1973). In contrast to the cytochrome P450-mediated activation of AA, conjugation with glucuronic acid and sulfate represent detoxication pathways of AA metabolism (Hinson, 1982). As phase I and phase II enzymes are involved in the bioactivation/detoxification of AA, we examined if CSC would change the toxicity of AA through modulation of metabolizing enzymes in primary cultured rat hepatocyte.

MATERIALS AND METHODS

Chemicals

CSC was gift from Dr. C. Gary Gairola (University of Kenturky, Tobacco and Health Research Institute, USA); the tar was prepared in a standard smoking machine from regular length, nonfilter cigarettes and was shipped by air mail.

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Preparation of rat hepatocytes and treatment

Hepatocytes were isolated from male Sprague-Dawley rats (200~250 g) by modification of perfusion method of Berry and Friend (Berry and Friend, 1969). Primary cultured hepatocytes were treated with CSC at concentration of 250 μg/ml for 2 days, and then incubated with AA (0.5 mM) for 24 hr. After final treatment cells were collected from the dish by scraping with a rubber policeman, and then stored at -70°C for enzyme assay. Protein was assayed by the method of Lowry (Lowry, *et al.*, 1951).

Enzyme assay

Pentoxyresorufin *O*-dealkylase (CYP4502B1) activity was assayed by a fluorometric measurement of resorufin formation, as described by Burke and Mayer (Burke and Mayer, 1974). Ethoxyresorufin *O*-dealkylase (CYP4501A1) and methoxyresorufin *O*-dealkylase (CYP4501A2) activities were measured exactly in the same manner as assay for CYP4502B1, except that the substrate concentrations utilized were 1.7 μM for ethoxyresorufin (Lubet *et al.*, 1985) and 5 μM for methoxyresorufin (Nerukar *et al.*, 1993), respectively. *N*-nitrosodimethylamine *N*-demetylase (CYP4502E1) activity was assayed based on the procedures described by Tu and Yang (Tu and Yang, 1983). Erythromycin *N*-demethylase (CYP4503A) activity was measured essentially in the same manner as CYP4502E1 assay by using 1 mM erythromycin as the substrate (Fabre *et al.*, 1988). UDP-glucuronyltransferase (UDP-GT) activity was determined by procedure of Isselbacher (Isselbacher *et al.*, 1962). Glutathion S-transferase (GST) activity of cytosolic protein was determined using the methods of Habig (Habig *et al.*, 1974). Phenol sulfotransferase (PST) was measured colorimetrically according to the method of Serkura and Jakoby (Sekura and Jakoby, 1979). Cytotoxicity of hepatocytes was quantitatively assessed by the measurement of LDH released into culture medium, using LDH assay kit.

Statistical Analysis

One-way analysis of variance was used to assess the statistical significance of changes in all indices, followed by Duncan's multiple range test, with $p<0.05$ set at the minimum level of significance by SPSS-PC+.

RESULTS

To estimate the effects of CSC on xenobiotic metabolizing enzymes, rat hepatocytes were treated with CSC for 2 days. CSC treatment resulted in a dramatic increase of CYP4501A1 activity to 7.5 fold of control in rat hepatocytes. Smaller but significant increase was observed with P4501A2. On the other hand, there were no changes in the activities of other phase I enzymes, CYP4502B1, CYP2E1, and CYP3A by CSC. The activities of phase II enzymes, GST, UDP-GT and PST were not affected by treatment of CSC in rat hepatocytes (Table I).

In order to investigate if the effects of CSC on the metabolizing enzymes could change the activation/detoxification of xenobiotics, we examined the effect of CSC on the toxification of AA, as model compound. Treatment with CSC for 2 days, up to concentration of 250 mg/ml, had not caused cytotoxicity (data were not shown). The significant increase of LDH release in media was not observed at concentration of 0.5 mM of AA. However, LDH release was markedly elevated by preteatment with CSC (250 μg/ml, 2 days) at non-cytotoxic concentration of AA (0.5 mM)(Figure 1).

DISCUSSION

In this study, we examined whether CSC could affect the activities of metabolizing enzymes *in vitro* system. The results of this study showed that CSC significantly increased the activities of CYP1A1 and CYP1A2 to 7.5 fold and 1.6 fold of control, and indicate that CSC can alter the metabolism of xenobiotics. Since these enzymes catalyze the activation of numerous protoxicants and procarcinogens, CSC may potentiate the activation of carcinogens such as heterocyclic aromatic amines, which would be present in tobacco smoke or foods at

Table I. Effect of CSC on the activities of metabolizing enzymes in rat hepatocytes[a,b]

Enzymes	Control	CSC (250 μg/ml)
1A1	4.16±0.62	31.24±5.9*
1A2	7.61±0.23	11.87±1.68*
2B1	0.31±0.10	0.23±0.05
2E1	0.92±0.18	0.80±0.22
3A4	13.39±1.31	16.99±2.17
UDP-GT	13.780±1.530	15.300±1.740
GST	6.080±0.560	5.660±0.500
PST	0.658±0.078	0.687±0.080

[a]Hepatocytes were treated with CSC (0 or 250 μg/ml) for 2 days. [b]Enzyme activities are expressed as follows: 1A1, 1A2, 2B1, resorufin (pmol) produced/min/mg protein; 2E1, 3A4, formaldehyde (nmol, pmol) produced/min/mg protein; UDP-GT, *p*-nitrophenol (nmol) consumed/min/mg protein; PST, β-naphthyl sulfate (nmol) formed/min/mg protein; GST, CDNB-GS (μmol) formed/min/mg protein. Values are expressed as mean±S.D. of six wells. *Significantly different from control at $p<0.05$

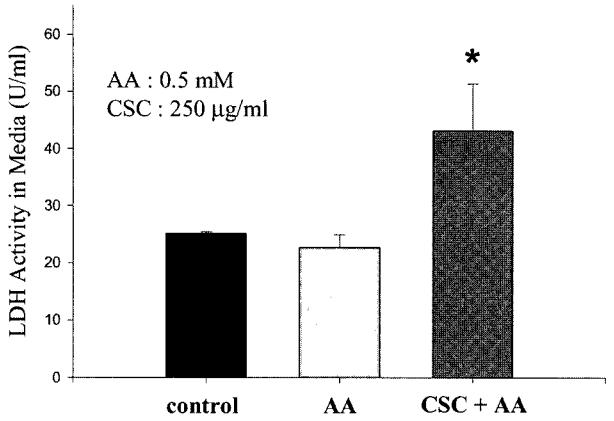

Fig. 1. Effect of CSC on Cytotoxicity of AA. After pretreatment of CSC (250 μg/ml, 2 days), hepato-cytes were treated with AA (0.5 mM) for 24 hrs. Cytotoxicity was measured by LDH activity in media. Values are expressed as mean±S.D. of six wells. *Significantly different from control at $p<0.05$

concentrations below their threshold for complete carcinogenic activity.

It is known that the hepatotoxic effects of AA are caused by *N*-acetyl-*p*-benzoquinoneimine (NAPQI) which produced by CYP450 enzymes (Dahlin *et al.*, 1984). The cytochrome P450s are a family of enzymes and it appears that the ethanol-inducible form, CYP4502E1 and the β-naphthofalvone-inducible form, CYP4501A2 are most effective in producing this reactive metabolite (Harvison *et al.*, 1988). In this study, CSC treatment increased the CYP4501A2 activity in rat hepatocytes, and CSC preteatment aggravated the cytotoxicity induced by AA. From these results it could be thought that CSC treatment increased the production of NAPQI through induction of CYP450 isozymes. The effects of CYP450 inducers such as 3-methylcholanthrene (3-MC) and phenobarbital (PB) on the hepatotoxicity of AA have been investigated extensively. In rats and mice, treatments of 3-MC and PB produce changes corresponding to increased toxicity of AA, including increased hepatocellular necrosis *in vivo* (Boyd and Bereezky, 1966). This effect could be explained by increase in toxic metabolites of acetaminophen with the consequence of enhanced CYP450s. Therefore in this study, the aggravating effect of CSC on AA toxicity can be explained by enhancing the formation of reactive metabolite of AA.

In conclusion, CSC increases the activities of CYP1A1 and CYP1A2, but have little effect on the phase II metabolozing enzymes in primary cultured rat hepatocytes. Moreover, potentiation of AA toxicity by CSC may be related to induction of P450s.

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