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Evidence for cytochrome P450 2E1 catalytic activity and expression in rat blood lymphocytes

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Abstract

Studies initiated to characterize cytochrome P450 2E1(CYP2E1) in freshly isolated rat blood lymphocytes revealed significant mRNA of CYP2E1 in control blood lymphocytes. RT-PCR studies have shown that as observed in liver, acute treatment of ethanol (single oral dose of 0.8 ml/kg b.wt , i.p), resulted in increase in the mRNA expression of CYP2E1 in freshly isolated rat blood lymphocytes. Western blotting studies using polyclonal antibody raised against rat liver CYP2E1 demonstrated significant immunoreactivity, comigrating with the liver isoenzyme, in freshly isolated control rat blood lymphocytes. Similar to that seen in liver, pretreatment of ethanol was found to produce an increase in the CYP2E1 isoenzyme in the blood lymphocytes. Blood lymphocytes were also found to catalyze the CYP dependent N-demethylation of N-nitrosodimethylamine (NDMA), which like in liver increased 2–3 fold following pretreatment of rats with known CYP2E1 inducers. Kinetic studies have further shown significant increase in the apparent Vmax and the affinity towards the substrate in rat blood lymphocytes indicating that as observed in liver, the increase in mRNA and protein expression following exposure to CYP2E1 inducers is associated with the increased catalytic activity of CYP2E1 in freshly isolated rat blood lymphocytes. The data indicating similarities of the blood lymphocyte CYP2E1 with the liver enzyme suggest that lymphocyte CYP2E1 levels in freshly isolated rat blood lymphocytes could be used to monitor tissue enzyme levels.

Keywords: Cytochrome P450 2E1; Blood; Liver; RT-PCR; Western blotting; Enzyme

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Introduction

Cytochrome P450 2E1 (CYP2E1) plays an important role in determining the toxicity of numerous environmental chemicals due to its capacity to metabolize solvents and other environmental procarcinogens to their cytotoxic and / or carcinogenic metabolites (Koop, 1992; Lieber, 1991). Xenobiotics such as ethanol, acetone, pyridine, pyrazole, isoniazid and pathophysiological conditions such as diabetes, fasting, obesity, high fat diet and long term alcohol consumption have been found to induce the expression of CYP2E1 in experimental animals and humans resulting in significantly enhanced toxicity (Raucy, 1995). CYP2E1 has also been shown to be involved in the increased incidence of liver disease and cancer in diabetics, alcoholics and obese individuals (Andersen et al., 1984; Koop, 1992; Lieber et al., 1979; Lieber, 1988, 1991; Raucy, 1995). Polymorphisms for the restriction enzymes Rsa 1 and Pst 1 have been detected in the 5'-upstream sequence of the gene encoding the human CYP2E1. These polymorphisms exhibit interethnic variation and may determine differences amongst the individuals towards the toxicity induced by xenobiotics (Boobis, 1992).

Monitoring of CYP2E1 levels in individuals exposed to environmental agents, known to be inducers of CYP2E1 or pathophysiologic conditions resulting in induction of CYP2E1 could help in the identification of individuals who may be at an increased risk. The use of metabolic markers like 6-hydroxylation of chlorzoxazone or N-1 and N-7 demethylation of caffeine may be used to assess *in vivo* CYP2E1 expression but these methods have disadvantages since these probes may not be solely metabolized by CYP2E1. Under certain circumstances or induction of other CYPs may occur which may contribute significantly to the reaction that is being monitored (Raucy, 1995; Streetman et al., 2000). Furthermore, the use of metabolic probes requires multiple sampling of urine and blood and an extended assessment period, usually in a hospital setting.

Instead of using metabolic markers to assess CYP2E1 *in vivo* levels, another alternative could be to use blood lymphocytes to monitor its expression provided CYP2E1 levels in lymphocytes parallel those in liver. CYP2E1 has been shown to be expressed in blood lymphocytes (Raucy et al., 1995, 1997; Scobbie and Mason, 1999; Soh et al., 1996; Song et al., 1990). However, most of these studies have been performed in microsomes isolated from cultured blood lymphocytes and which require addition of mitogens to stimulate CYP enzyme activities. Recent studies from our laboratory have shown that freshly isolated intact peripheral blood lymphocytes could be used as a tool to estimate CYP levels (Dey et al., 2001, 2002). These studies reported similarities in the expression and regulation of lymphocyte CYP1A1 with the liver isoenzyme (Dey et al., 2001). Freshly isolated intact rat blood lymphocytes were also found to catalyze CYP2E1 dependant lipid peroxidation and demethylation of N-nitrosodimethylamine, NDMA (Dey et al., 2002). To further investigate if the freshly isolated peripheral blood lymphocytes CYP2E1 could be used as a rapid screen to monitor hepatic changes, studies were initiated to investigate the similarities in the expression and regulation of CYP2E1 in blood lymphocytes with the liver isoenzyme.

Materials and methods

Chemicals

N-nitrosodimethylamine (NDMA), 3-methylcholanthrene (MC), phenylmethyl sulfonyl fluoride (PMSF), NADPH, bovine serum albumin (BSA), Histopaque 1077 and dithiothreitol (DTT) were