ROLE OF 5'FLANKING DNA FOR THE REGULATION OF CYP450IA1 GENE EXPRESSION

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To investigate the mechanism of the regulation of cytochrome P450IA1, the 5'-flanking region of a trout cytochrome P450IA1 was cloned into the CAT basic expression vector at HindIII site. This trout Cytochrome P450IA1 upstream DNA containing CAT construct was transfected into Hepa-1 cells. 3MC treatment to hepa I cells transfected with trout P450IA1-CAT construct increased CAT protein and mRNA by 2.81 fold when it was compared with that of control. This increase CAT protein and mRNA was decreased by concomitantly treated flavonoids and aminopyrine. The level of CAT protein was 29.2-58.0% of 3MC stimulated CAT protein.

INTRODUCTION

Multiple forms of cytochrome P450 exist; these enzymes are notable for their broad and overlapping substrate specificities. Although P450s usually mediate detoxification reactions, under some circumstances they activate their substrates to carcinogenic, mutagenic, and/or cytotoxic products. They also contribute to the oxidative metabolism of endogenous hormones, fatty acids, and cytokines. Some cytochrome P450 genes are expressed constitutively, while others (particularly those involved in xenobiotic metabolism) are inducible. In many cases, inducers are also substrates for the induced enzymes; therefore, P450 activities remain elevated only as needed. Enzyme induction usually enhances detoxification; thus, under most conditions, induction is a protective mechanism. Induction most often occurs at the level of transcription. Polycyclic aromatic hydrocarbons, such as the carcinogens 3-methylcholanthrene(3-MC) and benzo(a)pyrene, are prototypical inducers of several P450s, most notably P4501A1/1A2 and 1B1. Nuclear ''run-on'' experiments have revealed that induction primarily reflects an increase in

the rate of transcription of these genes, although posttranscriptional effects on P4501A2/1B1 have been reported. Our understanding of cytochrome P450 induction by aromatic hydrocarbons is based largely upon analysis of P4501A1 gene transcription. This study has been undertaken in order to gain further insight into the mechanism of the regulation of cytochrome P450 IAI by arylhydrocarbon.

MATERIALS AND METHOD

5'-flanking region P450 IA gene was clone into pCAT basic at HindIII site, and transformed into E.Coli HB101 Cells. Orientation of the cytochrome P450 IA gene was determined by restriction enzyme digestion. Correct oriented CAT construct was trnasfected into mouse Hepa I cells by either Calcium phosphate precipitation method or Lipofectin. In parallel experiment, mouse and human cytochrome P450IA CAT constructs were trnasfected into Hepa I cell culture medium for 48 hours, and 1nM of various flavonoids were added into cell culture medium 30 mimutes prior to 1nM 3MC addition.

After the treatments, cells were washed with phosphate buffered saline for 2 times and directly lysed by freeze and thaw for 3 cycles. Cell lysates were analyzed for total protein determination and for CAT protein determination by CAT ELISA, and for CAT mRNA was measured by RT-PCR.

RESULTS AND DISCUSSION

3MC treatment to hepa I cells transfected with trout P450-pCAT plasmid resulted 2.8 fold increase in CAT protein compared to hepa I cells without 3MC treatment. This data shows that trout cytochrome P450IA gene expression also could be regulated by 3MC and mouse transacting factor, 'arylhydrocaebon receptor could transactivate the fish cytochrome P450IA gene expression. This data also suggested that XRE might be present at the 5'flanking region of trout cytochrome P450IA. Flavonoids are known to inhibit cytochrome P450 enzymatic actions, but no study was carried out to see if flavonoids inhibit cytochrome P450IA gene expression. Therefore, we examined the effect of various flavonoids on CAT protein production and mRNA level of the Hepa I cells transfected with either mouse P450-CAT construct or trout P450CAT construct. 10^{-9} M Chrysin, 10^{-9} M Hesperetin, 10^{-9} M Kamepferol, 10^{-9} M Morin, 10^{-9} M Myrosetin, 10^{-9} M Aminopyrine showed no

effect on CAT protein of Hepa I cells transfected with P450CAT construct. However, 30 mimuits pretreatmrnat of each flabonoid(10^{-9}M) proir to 3MC (10^{-9}M) treatment, flabonoids deceased the CAT protein stimulared by 3 MC teatment. This data indecates flavonoid alone do not interact with cytochrome P450IA1 transcriptional and translational regulatory components, however, it interfere 3MC with interaction to transactivating complexes with cytochrome P450IA gene.

Likewise, mouse P450 CAT constuct transfected Hepa I cells responded to 3MC and flavonoid treatment with similar manner. RT-PCR was applied to measure the CAT mRNA in Hepa I cells transfected with cytochrome P450-CAT construct after the 3MC or 3MC plus flavonoids treatments. 3MC stimulated mRNA level of CAT over the control , but flavonoid treatment concomitantly with 3MC decreased the level of CAT stimulated by 3MC. This data showed flavonoids inhibit both CAT protein and mRNA that were stimulated by 3MC, which indicatd that flavonoid could inhibit the cytochrome P450IA gene expression at the level of transcription. These might be one of many mechanism of flavonoid action on regulation of cytochrome P450 action.