HIGH SUSCEPTIBILITY TO AFLATOXIN B₁ AND BENZO[A]PYRENE OF BALB3T3 A31-1-1 CELLS EXPRESSING MONKEY CYP1A1

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Accepted July 5, 1993

ABSTRACT — The monkey CYP1A1 has been expressed in BALB 3T3 A31-1-1 cells and the expressed proteins were assayed for their capacity to activate aflatoxin B₁ (AFB₁) and benzo[a]pyrene (B[a]P). The transformed cells were approximately 5.4- and 4.7-fold more sensitive to AFB₁ and B[a]P than the parental cells, respectively. These results indicate that monkey CYP1A1 cDNA encoded a functional protein and that the expressed CYP1A1 enzyme is active for the activation of B[a]P as well as AFB₁ to produce toxic metabolites.

KEY WORDS: CYP1A1, BALB3T3 A31-1-1, Benzo[a]pyrene, Cytotoxicity.
demonstrated that MKahl had the capacity to activate aflatoxin B₁ (AFB₁) metabolically in the stable expression system of Chinese hamster CHL cells. CHL cells expressing MKahl, but not parental CHL cells, revealed sensitive response to AFB₁ in an assay for mutagenicity at the hypoxanthine-guanine phosphoribosyltransferase (HGRPT) locus. However, the cells expressing monkey CYP1A1 did not metabolize B[a]P.

In this study, we tried to introduce monkey CYP1A1 cDNA into BALB3T3 A31-1-1 cells (JCRB0601, provided by the Japanese Cancer Research Resources Bank) to investigate whether or not the BALB3T3 A31-1-1 cells expressing MKahl showed the elevated cytotoxicity of AFB₁ and B[a]P compared with the parental BALB3T3 A31-1-1 cells.

The full-length monkey CYP1A1 cDNA was ligated with pUC-SRα vector consisting of the SV40 early promoter and the R segment and part of the U5 sequence of the long terminal repeat (LTR) of HTLV-1. The resultant plasmid, pUC-SRα/MKahl (Fig. 1), was co-transfected with the plasmid pSTneoB (Katoh et al., 1987) into BALB3T3 A-31-1-1 cells by the method of calcium phosphate precipitation (Graham and van der Eb, 1973). After 10 days of incubation, ten G-418 resistant colonies were propagated.

![Fig. 1 Structure of monkey CYP1A1 expression vector, pUC-SRα/MKahl. The cDNA of pUC-SRα/MKahl covers the entire coding region for monkey CYP1A1 and is preceded by SRα promoter consisting of SV40 early promoter, the R segment and the part of the U5 sequence of the long terminal repeat (LTR) of HTLV-1.](image-url)

To obtain a clone expressing monkey CYP1A1 mRNA, total RNAs from each clone were prepared (Chirgwin et al., 1979). Subsequently, RNA blot analysis was performed (Church and Gilbert, 1984). As shown in Fig. 2, only one clone, L7, out of ten clones was found to express monkey CYP1A1 mRNA (lane 1). The parental cells, BALB3T3 A-31-1-1 cells, did not contain hybridizing mRNA (lane 2). The size of the mRNA detected in L7 cells was about 2.1 kb.

AFB₁ and B[a]P are known to be activated metabolically to mutagens. In this study, L7 and BALB3T3 A-31-1-1 cells were exposed to AFB₁ and B[a]P dissolved in dimethylsulfoxide (0 to 0.5 μg/ml) for 3 days. Subsequently, the cells were washed twice with phosphate-buffered saline (PBS) and maintained in the culture medium alone. Six days later, the medium was removed and the cells attached to the culture dishes were rinsed with saline, fixed with 3% formaldehyde and stained with 0.1% crystal violet solution.
Expression of monkey CYP1A1 cDNA

The Japanese Society of Toxicology

Figure 3: Cytotoxicity of (A) AFB_1 and (B) B[a]P in BALB3T3 A31-1-1 and L7 cells. Cells were exposed to AFB_1 and B[a]P for 3 days and survival was measured. Open circles, BALB3T3 A31-1-1 cells; closed circles, L7 cells.

Subsequently, the colonies were counted. The results are shown as a percentage of control obtained with the solvent alone without promutagens. The sensitivities to AFB_1 and B[a]P to show cytotoxicity of L7 and BALB3T3 A-31-1-1 cells were compared (Fig. 3A and B). The LD_{50} values of AFB_1 and B[a]P were as follows: 0.056 and 0.015 μg/ml in L7, and 0.30 and 0.07 μg/ml in BALB3T3 A-31-1-1 cells, respectively. Thus, L7 cells were 5.4 and 4.7 times more sensitive to AFB_1 and B[a]P than BALB3T3 A-31-1-1 cells, respectively. The cytotoxicities of AFB_1 and B[a]P in L7 cells were abolished with α-naphthoflavone which was known to be a specific inhibitor of CYP1A (data not shown).

In the present study, we showed that L7 cells were more sensitive to B[a]P, in addition to AFB_1, than parental cells, indicating that monkey CYP1A1 possessed the capacity of metabolic activation for B[a]P. On the major activation pathway of B[a]P, the following has been proposed; (i) B[a]P is primarily converted into (+)-B[a]P-7,8-epoxide by CYP1A. (ii) This metabolite is further transformed into (−)-trans-B[a]P-7,8-diol by epoxide hydrolase. (iii) Resultingly, (−)-trans-B[a]P-7,8-diol is changed into (+)-anti-B[a]P-7,8-diol-9,10-epoxide by CYP1A. (iv) This compound binds to DNA to form covalent adduct (Weinstein et al., 1976; Jeffrey et al., 1977). Accordingly, CYP1A and epoxide hydrolase are indispensable to activate B[a]P.

Therefore, BALB3T3 A-31-1-1 cells may possess epoxide hydrolase in contrast to yeast and CHL cells. This postulation does not conflict with the report by Cortesi et al. (1983) who demonstrated that addition of B[a]P caused BALB3T3 A31-1-1 cells to transform.

We have already established CHL cells carrying monkey CYP1A1 and guinea pig cytochrome P450 reductase (Sawada et al., 1993). These cells were more sensitive to AFB_1 than CHL cells carrying monkey CYP1A1 cDNA (A-15). Although parental CHL cells possessed P450 reductase activity, the level of the P450 reductase was assumed to be insufficient for A-15 to show the full activity of CYP1A1. It is possible that L-7 cells will become more sensitive to B[a]P if the P450 reductase cDNA is introduced to L-7 cells. This hypothesis is under current experiment in this laboratory.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan, and the Suhara Foundation (Japan).

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