The role of CYP1A1 in glycogen depletion of Dioxin-induced wasting syndrome and the development of CYP1A1-IRES-connected luciferase method

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1. INTRODUCTION

Dioxin cause many toxic effects, and unraveling the mechanisms may be applied to the risk assessment of other chemicals causing same symptoms. Additionally, reporter assay can show the dose-response curve which is the basis of toxicology easily, and may be used to evaluate the different susceptibilities between species by modification. The purposes of this thesis are to unravel the mechanism of glycogen depletion of dioxin-induced wasting syndrome and to develop a new method, IRES-connected luciferase system.

2. UNRAVELING THE MECHANISM OF GLYCOGEN DEPLETION OF DIOXIN-INDUCED WASTING SYNDROME

Wasting syndrome is the depletion in body weight while being exposed to the lethal dose of dioxin, during when decrease in glycogen can be observed. By literature research, the glycogen depletion was hypothesized to be resulted from the stimulation of heme synthesis and acceleration of TCA cycle due to heme depletion caused by the expression of cytochromeP450s. At the beginning, cells were exposed to β-naphthoflavone, which is an AhR ligand as dioxins, and glycogen depletion was observed over time. Additionally, it was found that the CYP1A1 activity correlated with the depletion rate of glycogen by comparing human, mouse and rat hepatoma cells. Next, inhibiting the hypothetic pathway by RNA interference of aminolevulinic acid synthetase which connects heme synthesis and TCA cycle, the glycogen did not decrease despite the exposure to β-naphthoflavone.

3. THE DEVELOPMENT OF NEW METHOD, IRES-CONNECTED LUCIFERASE SYSTEM

An IRES-connected luciferase system, which may express in vitro transcription,, was developed by using IRES sequence and modifying reporter assay system ordinarily used. CYP1A1 may be a biomarker of dioxin toxicity, and IRES-connected luciferase system using various CYP1A1 deletions caused intron splicing precisely. In addition, luciferase activity declined as the CYP1A1 gene length increased in IRES-connected luciferase system, but the construct with first intron showed lower luciferase activity than the construct without first intron, despite the length of CYP1A1 gene was same.

4. CONCLUSION

In vitro assay of pathway inhibition revealed that the glycogen depletion by AhR ligand occurred through heme synthesis and TCA cycle. In addition, the CYP1A1 activity correlate with the rate of glycogen depletion.

IRES-connected luciferase system express the transcription regulatory mechanism in vivo, and revealed that the first intron internal sequence of CYP1A1 gene, as dioxin toxicity biomarker, has a slight suppression status of transactivity.