PCB153 INCREASES PROLIFERATION AND SHORTENS G1 PHASE OF EGF-STIMULATED PRIMARY RAT HEPATOCYTES

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Introduction

Whereas it has been established that the toxicities of dioxins and dioxin-like polychlorinated biphenyls (PCB) depend upon binding to the aryl hydrocarbon receptor, the toxic effects of non-dioxinlike PCB (ND-PCB) remain largely unexplained. Studies have shown that exposure to the ND-PCB congener PCB153 alters liver gene expression, and also induce liver proliferation (1, 2). The carcinogenic potential of PCB153 remains unresolved. In this project we seek to establish a robust model for identification of molecular mechanisms involved in the toxicity of ND-PCBs. PCB153 was chosen since it is one of the most abundant ND-PCB congener, and has shown definitive effects in several animal models (2-4).

Methods

A primary rat hepatocyte culture model was adapted (5). Isolated rat hepatocytes were grown in a synthetic medium containing insulin and dexamethasone, but not growth factors. PCB153 at different concentrations was added to the medium 3 hours after hepatocyte plating. 10 nM EGF was added to the medium 24 hours thereafter. Tritiated thymidin incorporation was measured to define effects on proliferation, whereas LDH concentration in the medium was used as a parameter of cell death.

Results and Discussion

Results showed that PCB153 in the 3-30 μM concentration range increased the proliferative effect of EGF in a dose-dependent manner (fig. 1). Further studies demonstrated that the increased proliferation was accompanied by a shortened G_1- time (fig. 2). Whereas PCB153-exposed cells had entered DNA synthesis 18 hours after EGF stimulation, control cells showed S-phase entry 28 hours after EGF stimulation. Increased cell death was not observed at the applied PCB153 concentrations (fig. 3). These results suggest that PCB153 exposure alters the growth factor regulation of hepatocyte proliferation.

References

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Figures

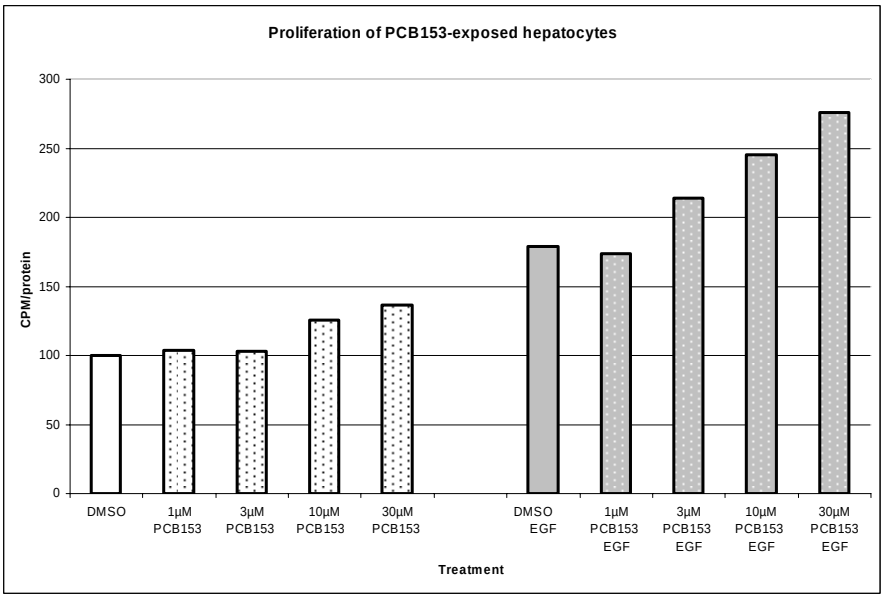

Fig. 1

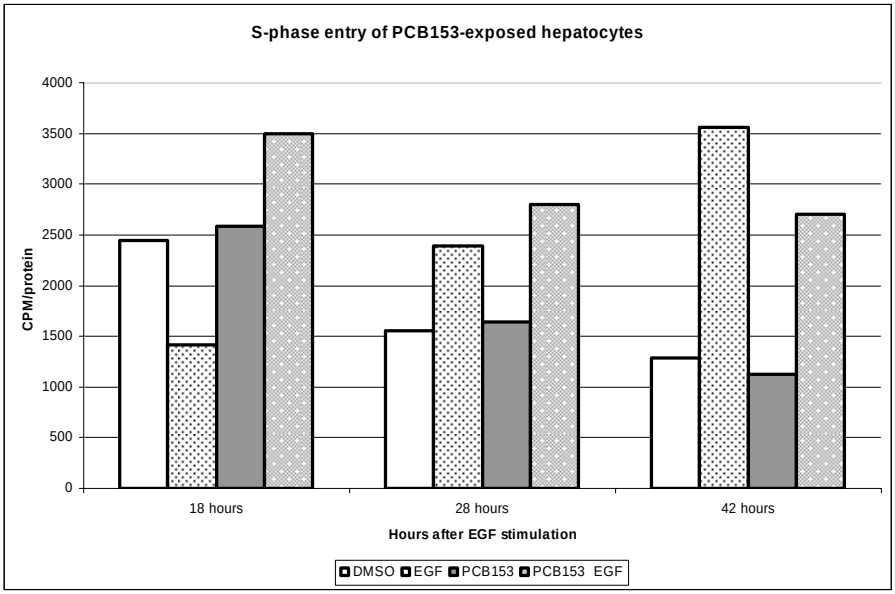
S-phase entry of PCB153-exposed hepatocytes
CPM/protein
4000
3500
3000
2500
2000
1500
1000
500
0
18 hours
28 hours
42 hours
Hours after EGF stimulation
DMSO
EGF
PCB153
PCB153 EGF

Fig. 2

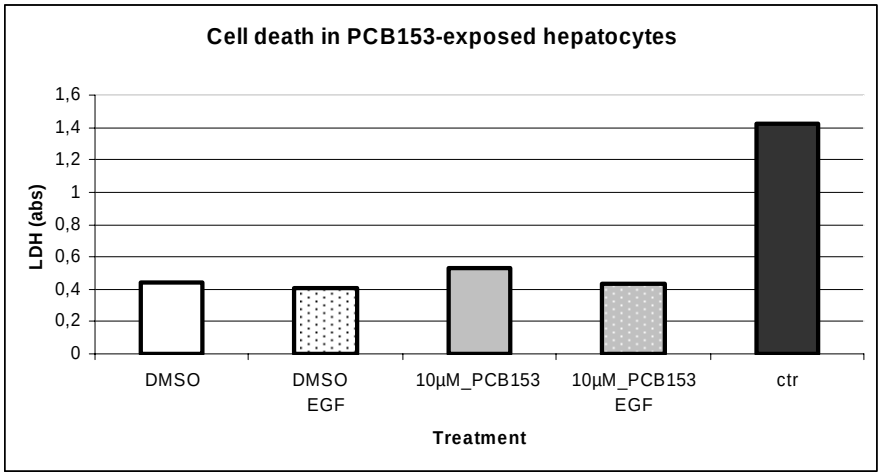
Cell death in PCB153-exposed hepatocytes
LDH (abs)
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
DMSO
DMSO EGF
10µM_PCB153
10µM_PCB153 EGF
ctr
Treatment

Fig. 3

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