EFFECTS OF UV IRRADIATION ON THE FORMATION OF NUCLEAR Ah RECEPTOR COMPLEXES IN MOUSE HEPA 1C1C7 CELLS

T.R. Narasimhan, X. Wang and S. Safe*

Department of Veterinary Physiology and Pharmacology

College of Veterinary Medicine, Texas A&M University

College Station, TX 77843

ABSTRACT

Irradiation of mouse Hepa 1c1c7 cells with light > 300 nm for up to 15 minutes did not significantly decrease the number of viable cells as assessed by the trypan blue dye exclusion method. However, after incubation of the irradiated cells with [^{3}H]-TCDD for 3 hours it was apparent that the formation of nuclear receptor complexes was dependent on the length of irradiation. The levels of nuclear Ah receptor levels isolated from cells irradiated $\leq$ 2 minutes were $\geq$ 80% of the levels in non-irradiated cells; however, irradiation for $\geq$ 4 minutes resulted in a > 50% decrease in nuclear Ah receptor levels.

INTRODUCTION

Photoaffinity labeling of specific ligands to their corresponding receptor or acceptor macromolecules is a technique which has been extensively utilized in the biomedical sciences (1). The *in vitro* photoaffinity labeling of both the cytosolic and nuclear Ah receptor have recently been reported using extracts from mouse liver cytosol or mouse Hepa 1c1c7 cell nuclei respectively (2,3). This report described results from preliminary studies on the irradiation of suspensions of Hepa 1c1c7 cells treated with [^{3}H]-2,3,7,8-TCDD and the in situ formation of the photolabeled nuclear Ah receptor complex.

MATERIALS AND METHODS

Cell Growth and Irradiation

Hepa 1c1c7 cells were grown as a continuous cell line in α-minimum essential medium (α-MEM) supplemented with 10% fetal calf serum. After reaching 90% confluency, the cells were harvested by trypsinization and washed in the Hank's balanced salt solution. Total cell counts and viable cell number were assessed by the trypan blue dye

exclusion method using a hemocytometer. The washed cells were suspended in α-MEM (3 x 10^6 cells in 5 ml) and distributed into quartz ampoules (0.6 x 24 cm i.d.) and irradiated for different time periods with a mercury-arc lamp (450 watts) filtered by a pyrex immersion well to give light intensity at wavelengths > 300 nm. At the end of each time point total cell counts and viable cell numbers were determined.

Irradiated cells from each ampoule were transferred into 25 cm^2 tissue culture flasks, incubated for 3 hours with [^{3}H]-TCDD (2 nM final concentration) and actinomycin-D (10 μM). Treatment of the cells with radioligand and a 200-fold excess of 2,3,7,8-tetrachlorodibenzofuran (TCDF) gave the non-specifically bound values. Incubated cells were washed with PBS (pH 7.6, 0.9% NaCl) and HEGD buffer (pH 7.6, 25 mM Hepes, 1.5 mM EDTA, 10% glycerol and 1 mM molybdate) and incubated for 1 hr at 4° C in the same buffer containing 0.5 M KCl. The nuclear extracts were obtained after centrifugation at 105,000 x g for 1 hour (2° C). Unbound and loosely bound [^{3}H]-TCDD were removed by treating with dextran-coated charcoal.

RESULTS AND DISCUSSION

Figure 1 illustrates that after irradiation of wild-type Hepa 1c1c7 for up to 15 minutes, the total cell counts (determined by trypan blue dye exclusion) were similar at all time points less than 15 minutes and only slightly decreased after 15 minutes. In contrast, when the irradiated cell suspensions were incubated with [^{3}H]-TCDD there was a time-dependent decrease in the accumulation of nuclear Ah receptor complexes. Thus, although UV irradiation had minimal effects on the viable cell counts it was apparent that photolysis of the cells resulted in impairment of some of the processes associated with the formation of nuclear receptor complexes. Preliminary studies indicate that photolabeled nuclear Ah receptor complexes are formed when the cells are irradiated for short time periods (1-2 minutes) and the properties of the specifically bound photolabeled nuclear protein were similar to those which have previously been described (3).

ACKNOWLEDGEMENTS

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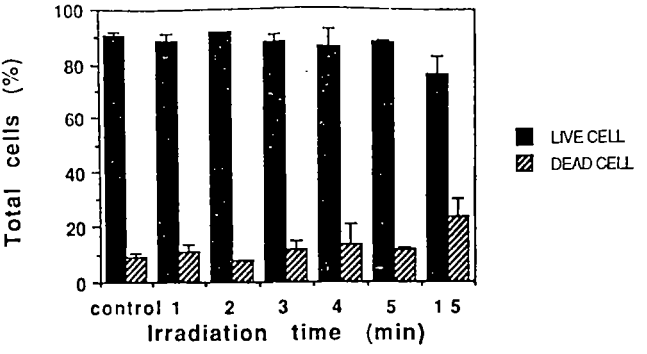

Figure 1. Time-Course Effects of UV Irradiation on Viable Cell Counts.

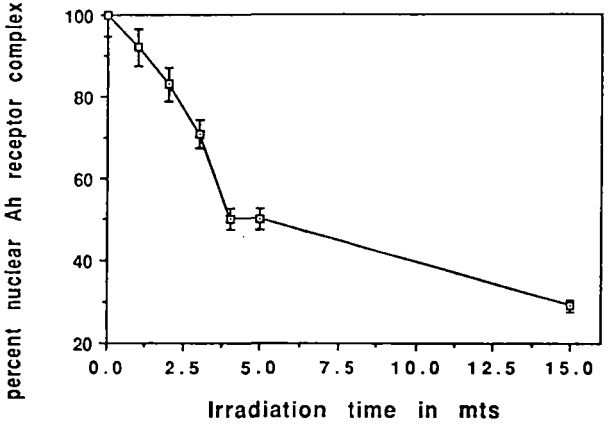

Figure 2. Effects of UV Irradiation on the In Situ Formation of Nuclear Ah Receptor Complexes in Hepa 1c1c7 Cells.

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