

Ethanol-Induced Defects on Zebrafish Retinal Development: Rescue by Nutritional Supplements

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Fetal Alcohol Spectrum Disorder (FASD), a result of prenatal alcohol exposure, produces a wide range of developmental defects including severe ocular defects that include microphthalmia, optic nerve hypoplasia, scotopic vision loss and coloboma. The zebrafish FASD model recapitulates many defects seen in human patients. Ethanol exposure (100 and 150 mM) during early development (midblastula transition through somitogenesis, 2-24 hours post fertilization, hpf) produced severe ocular defects including microphthalmia, optic nerve hypoplasia and photoreceptor differentiation defect. Examining specific terminal differentiation markers showed ethanol-induced defects in differentiation of most retinal cell types. Ethanol exposure altered gene expression of critical transcription factors. Increased cell death accounted for the small eye phenotype, and the retina responds with increased proliferation in the outer nuclear layer, inner nuclear layer, and ciliary marginal zone (CMZ). Ethanol treated retinas showed an expanded CMZ and cell cycle exit defects of the photoreceptor cells. In order to examine progenitor cell populations and differentiation defects in the ethanol treated retinal cells, specific markers for retinal stem, precursor and progenitor cell populations were examined. While control retinas showed terminally differentiated photoreceptors at 72 hpf, ethanol treated retinas expressed immature and nascent photoreceptor markers in cell populations undergoing proliferation. Nutrient co-supplement with retinoic acid (RA) or folic acid (FA) with ethanol during 2-24 hpf rescued photoreceptor differentiation and optic nerve defects. Competitive inhibition of RA synthesis by ethanol was hypothesized by Duester (1991), and rescue of ethanol-induced retinal defects suggest an effect on RA levels in the developing retina. Treatment with RA inhibitors produced retinal defects similar to ethanol-treated embryos. Interestingly, RA supplementation (24-48 hpf and 48-72 hpf) following ethanol treatment (2-24 hpf) restored photoreceptor differentiation suggesting RA provides a critical signal for precursor cell differentiation. In contrast, post-treatment with FA, did not restore retinal cell differentiation. FA functions as a critical component of one-carbon metabolism and can influence histone- and DNA-methyl transferase activities. Molecular mechanisms underlying disruption of cell cycle exit and FA rescue of ethanol-induced defects are being actively studied.