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Age-dependent sensitivity of Big Blue transgenic mice to the mutagenicity of *N*-ethyl-*N*-nitrosourea (ENU) in liver☆

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Abstract

The incidence of childhood cancer is increasing and recent evidence suggests an association between childhood cancer and environmental exposure to genotoxins. In the present study, the Big Blue transgenic mouse model was used to determine whether specific periods in early life represent windows of vulnerability to mutation induction by genotoxins in mouse liver. Groups of mice were treated with single doses of 120 mg *N*-ethyl-*N*-nitrosourea (ENU)/kg body weight or the vehicle either transplacentally to the 18-day-old fetus or at postnatal days (PNDs) 1, 8, 15, 42 or 126; the animals were sacrificed 6 weeks after their treatment. The *cII* mutation assay was performed to determine the mutant frequencies (MFs) in the livers of the mice. Liver *cII* MFs for both sexes were dependent on the age at which the animals were treated. Perinatal treatment with ENU (either transplacental treatment to the 18-day-old fetus or i.p. injection at PND 1) induced relatively high MFs. However, ENU treatment at PNDs 8 and 15 resulted in the highest mutation induction. The lowest mutation induction occurred in those animals treated as adults (PND 126). For instance, the *cII* MF for the PND 8 female group was 646×10^{-6} while the MF for female adults was only 145×10^{-6}, a more than 4-fold difference. Molecular analysis of the mutants found that A:T → T:A transversions and A:T → G:C transitions characterized the pattern of mutations induced by ENU in both the neonate and adult mice, while the predominate type of mutation in the controls was G:C → A:T. The results indicate that mouse liver is most sensitive to ENU-induced mutation during infancy. This period correlates well with the age-dependent sensitivity to carcinogenicity in mouse liver, suggesting that mutation is an important rate-limiting factor for age-related carcinogenesis.

Keywords: Ethylnitrosourea; Age; Neonate; Mutagenicity; Transgenic mice; Sensitivity

Abbreviations: ENU, *N*-ethyl-*N*-nitrosourea; PNDs, postnatal days; MF, mutant frequency; DMSO, dimethylsulfoxide

☆ The views presented in this article do not necessarily reflect those of the Food and Drug Administration.

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1. Introduction

The protection of children from environmental toxins is a major challenge to modern society because children can be more vulnerable due to their unique exposures and susceptibilities. Childhood exposure to environmental chemicals may contribute to or exacerbate certain chronic and disabling diseases like cancer [1]. Although the causes of cancer in newborns and infants remain unknown, environmental exposure to genotoxins during the perinatal period and susceptibility to these toxins during early development are strong possibilities [2]. Data from the National Cancer Institute's Surveillance Epidemiology and End Results program suggest that the annual incidence of childhood cancers is increasing and about 12% of the cancer cases were tumors acquired during infancy [3–5].

The mouse model has been used to evaluate factors modifying the carcinogenic response of various tissues to different types of chemical carcinogens. The age at which animals are exposed is the most effective modulator of carcinogenesis in liver and lung, as well as some other tissues. Infancy appears to be the most susceptible period for carcinogenesis in a great variety of tissues [6]. Exposure of newborn ($\leq$24-h-old) and neonatal ($\leq$3-week-old) mice to only a few doses of any one of a number of genotoxic chemical carcinogens results in tumors approximately one year after the treatment. The primary sites of tumor induction are the liver and the lung. For example, polycyclic aromatic hydrocarbons usually do not induce liver cancer when administered to young adult mice, but do so when given to newborn and neonatal animals [7].

Mutations play an important role in the etiology of cancer. Newborn and neonatal mice are highly sensitive to carcinogens that exert tumorigenicity through a genotoxic mechanism [8–11]. Therefore, it is reasonable to suspect that these animals also possess higher sensitivity to the mutagenicity of genotoxins than do adult animals. However, studies on age-related chemical mutagenicity are rare because mutagenesis studies are often conducted using in vitro tests or in vivo tests in which endogenous genes are utilized. Assays that test mutations in endogenous genes often require testing rapidly dividing tissues like lymphocytes, and may not have the capability to detect the effect of age because cells in these tissues are rapidly dividing regardless of age [12]. The recently developed Big Blue transgenic mutation models represent a novel approach for studying mutant frequencies (MFs) and types of mutations in nearly all tissues, thus permitting the direct comparison of cancer incidence with MF [13–15]. In these systems, the chromosomally-integrated λLIZ/*lacI* or *cII* gene is used as the target for mutation. Following recovery of the transgene from the tissue(s) of choice, the target sequence is packaged into phage particles, bacteria are infected, and mutants are identified and quantified. In present study, we treated different age Big Blue transgenic mice with *N*-ethyl-*N*-nitrosourea (ENU) using a carcinogenesis protocol to explore the age-related mutagenic sensitivity in liver and its relationship to carcinogenesis.

2. Materials and methods

2.1. Animals and treatments

The recommendations set forth by our Institutional Animal Care and Use Committee for the handling, maintenance, treatment, and sacrifice of the animals were followed. Pregnant female Big Blue C57BL/6 transgenic mice were purchased from Taconic Laboratories (Germantown, NY). These mice had been bred with non-transgenic C3H male mice by the supplier. The $B6C3F_1$ offspring were separated according to sex, pooled, and then distributed to individual litters during the first week following birth. Six to eight same-sex pups were assigned to each foster mother. The ENU was purchased from Sigma (St. Louis, MO) and administered in dimethylsulfoxide (DMSO). Animals were given a single i.p. injection of 120 mg ENU/kg body weight or vehicle control in a volume of 2 ml/kg body weight at various times during their development. For the prenatal treatment groups, five pregnant mice were treated at 18 days of gestation, and at birth the pups were pooled and assigned randomly to the mothers. For the postnatal treatment groups, the pups were pooled and distributed randomly into five groups at birth. Male and female mice received treatment at postnatal days (PNDs) 1, 8, 15, 42, or 126. For male mice, there were 3 concurrent controls. Control 1 was administered DMSO at PND 8 to serve as a control for the prenatal, PNDs 1, 8 and 15 treatment groups. Controls 2 and 3 were treated with DMSO at PNDs 42 and 126 to serve as con-