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Physiologically based toxicokinetic modeling of secondary acute myelolytic leukemia

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ABSTRACT

Benzene, designated as environmental and occupational carcinogen and hematotoxin, has been associated with secondary leukemia. To develop a toxicokinetic model of AML, benzene can be used as leukemogenic agent. The aim of the present study was to optimize the dose, period and time of cumulative benzene exposure of Swiss Albino mice and to analyze survival rate; alteration in cell cycle regulation and other clinical manifestations in mice exposed to benzene vapour at a dose 300 ppm × 6 h/day × 5 days/week for 2 weeks, *i.e.*, 9000(a) ppm cumulative dose. Analyzing physiological parameters like plasma enzyme profile, complete hematology (Hb %, RBC indices and WBC differentials), hematopoietic cells morphology, expression of cell cycle regulatory proteins, tissue histology and analysis of DNA fragmentation, optimum conditions were established. Down regulation of p53 and p21 and up regulation of CDK2, CDK4, CDK6, cyclin D1 and E in this exposed group were marked as the optimum conditions of cellular deregulation for the development of secondary AML. Elevated level of Plasma AST/ALT with corresponding changes in liver histology showing extended sinusoids within the hepatocytic cell cords in optimally exposed animals also confirmed the toxicokinetic relation of benzene with leukemia. It can be concluded from the above observations that the 9000(a) ppm exposed animals can serve as the induced laboratory model of secondary acute myeloid leukemia.

1. Introduction

The availability of appropriate disease model system is crucial for development of effective treatment protocol. Animal models are an approximation of reality and their use in developing anticancer drugs is still controversial. Two biomedical models of neoplasia are used for preclinical testing, like spontaneous environmental model of carcinogenesis and disease model of transplantation. Different models, like spontaneous, oncogenic, chemical, ultraviolet exposed, and viral infected models, defined many aspects of carcinogenesis progression and therapeutic intervention (McCormack et al., 2005; Zuber et al., 2009; Hagenbeek and Martens, 1991; Beurlet et al., 2013). Though spontaneous environmental models have significant role in biomedical research, the long period of latency for most of these models makes them impractical for preclinical studies of tumor modulation. There are many commercially available human and murine cell lines for testing tumorigenicity in mice (Price et al., 1990; Al-Hajj et al., 2003; Singh et al., 2004; Ricci-Vitini et al., 2007). These tumorigenic cell lines are generally easy to maintain, selectable for distinctive mutations *in vitro* and of *in vivo* behavior of immunodeficient, immunosuppressed, humanized and immunocompetent strains of mice (McCune et al., 1988; Mosier et al., 1988, 1991; Kamel-Reid and Dick, 1988;

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Dick et al., 1991; Mombaerts et al., 1992; Shinkai et al., 1992; Mule and Jicha, 1992). But specific features suitable for transplantation of these tumor cell lines may affect experimental design because of the need for specific mouse strains of specific sex, and altered host immunity. Furthermore, *in vitro* spontaneous mutations that allow selection of tumor cell lines with unique behaviors can also result in point mutations that lead to changes in histology, sensitivity, and behavior of these tumors *in vivo*. Since the field of biomedical research is rapidly changing, a thorough knowledge on this area is warranted for selection of the most appropriate models for any therapeutic development program (Cormack et al., 2005).

Cancer induction is a multistep process requiring interaction and mutation of the genomic DNA followed by the selective clonal proliferation of the mutated cell and there are additional genetic changes during proliferation leading to neoplasia. Benzene is a ubiquitous environmental chemical that cause different types of haematotoxicity, like acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and hematological cancers (Smith, 1996a,b; Snyder et al., 1980; Cronkite et al., 1984, 1989; Kawasaki et al., 2001; Yoon et al., 2001). Tumors reported in mice exposed to benzene include different types of lymphoma, neoplasm and carcinoma (Cronkite, 1986; Huff et al., 1989; Maltoni et al., 1989; Farris et al., 1993). Acute myeloid leukemia (AML) is a common and deadly form of leukemia. The *in vivo* modeling of human AML has been dominated by the use of mouse and most of the genetic alterations have been modeled in mouse exposed at a particular dose of benzene vapour (Aksoy et al., 1974; Curt et al., 1994; Rabble and Wong, 1996; Natelson, 2007). The mode of action for benzene can be proposed to base on the sequelae to involve the key events like, metabolism of benzene in the body, interactions of the benzene metabolites with target cells in the bone marrow, formation of the mutated target cells in the bone marrow, the selective clonal proliferation of these mutated cells and formation of neoplasm (leukemia) (Wolman, 1977; Subrahmanyam et al., 1990; Eastmond et al., 1987; Smith et al., 1989; Chen and Eastmond, 1995; Yoon et al., 2003). Quantification of the key events and modifying factors are challenging and the generation of a biologically based risk model for risk assessment purposes will require additional mechanistic research. Exposure of mice to benzene vapour induces inflammatory responses, oxidative stress, alterations in cell cycle progression and DNA damage including DNA strand breakage; mitotic recombination; chromosome translocation and aneuploidy, which together have been implicated in various blood diseases, including the development of acute myeloid leukemia (Aksoy et al., 1974; Snyder et al., 1980). So, epigenetic effects of benzene metabolites on the bone marrow stroma and perhaps the stem cell itself may then faster the development and survival of the leukemic clone (Green et al., 1981; Cronkite et al., 1982; Abraham, 1996; Hirabayashi and Inoue, 2010). In this regard the benzene exposed animal model can act as a significant template for the study of environmental–occupational cancers with respect to different parameters measuring clinical prevention intervention and this physiologically based toxicokinetic models can provide more accurate estimates of the risks by predicting tissue exposure to the active compounds.

2. Materials and methods

2.1. Chemicals

The primary antibodies specific for cyclin D1, cyclin E, CDK2, CDK4, CDK6, Cip1/p21, p53 and β-actin; the secondary antibody (anti-rat conjugated), protease inhibitor cocktail, AST/ALT assay kit purchased from Sigma Aldrich Chemical Private Ltd., USA DNA isolation kit was purchased from Bangalore Genei, India and Lymphocyte isolation media was from HiMedia. All other chemicals used in our experiments were of analytical grade mentioned otherwise.

2.2. Animals

Six to eight weeks old Swiss albino male mice (*Mus musculus*) from specified strain were purchased from a reputed supplier at regular basis and were maintained in stainless steel wire cages (Tarsons, India) under 12-h light:12-h dark cycle. Pellet diet (West Bengal Diary and Poultry development corp. Ltd., Kalyani Industrial Area, Kalyani) was provided *ad libitum* except during the daily period of benzene inhalation. Water was supplied *ad libitum* automatically through the tubing throughout the study.

2.3. Benzene exposure

Benzene (MERCK, India) vapour was generated by heating liquid benzene (HPLC grade) at 16 °C and channelled into the inhalation chamber. The groups of experimental mice, each group containing 5 mice were exposed 100 ppm of benzene for 6 h/day, 5 days/week for 2 weeks, 300 ppm of benzene for 6 h/day, 5 days/week for 2 weeks, 3 weeks, 4 weeks and in another group of study 150 ppm × 12 h/day × 5 days/week; 150 ppm × 10 h/day × 6 days/week and 200 ppm × 5 h/day × 3 days/week (Fig. 1) in 1.3 m^3 inhalation chambers (S.B. Equipments, W.B., India) and one control group against each experimental group containing 5 mice was exposed to ambient air for the same time duration, interval and periods. Benzene concentration in the chambers was monitored at half an hour intervals during daily exposures. Cumulative exposure was measured as ppm × no. of hour × no. of days. The temperature and humidity in the chamber were automatically maintained at 24 ± 1 °C and 55 ± 10% respectively. Mice were observed for their survival study after stopping the exposure.

2.4. Blood and bone marrow parameters

Peripheral blood was collected by heart puncture method. One part of collected blood was used to evaluate total and differential count of leukocytes (WBC) and red blood cells (RBC) numbers by Neuber hemocytometer (Sharma and Pandey, 2010) using diluting solution. Hemoglobin concentration was measured with the help of Sahil's hemoglobinometer (Balasubramaniam and Malathi, 1992). Other blood parameters including platelet count and analysis of RBC indices such as hematocrit, MCV, MCH, MCHC were performed from control and treated animal groups according to Sarma (1990). Another part of blood was used to draw smear which was stained using