PROTOCOL FOR MOLECULAR DOSIMETRY STUDIES ON VINYL CHLORIDE

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Listed below is a series of studies on the formation and repair of DNA adducts induced by vinyl chloride (VC) that will provide a vastly improved understanding of the mechanisms of vinyl chloride carcinogenesis. These studies will provide information relevant to cancer risk assessments of vinyl chloride. The studies would be conducted in a manner that will complement the two generation reproductive and developmental toxicity studies that will be conducted at Huntingdon Life Sciences Laboratory.

Dose-response Studies

Presently, we have data showing that preweanling rats are more susceptible than adults to VC carcinogenesis, that preweanlings develop 3-fold greater numbers of VC DNA adducts, and that preweanlings have 3-fold higher expression of CYP 2E1. The adduct and carcinogenesis data are all from "high" exposures of ~500 ppm. No information exists on lower exposures. In addition, we have clearly demonstrated that DNA adducts identical to those formed by VC are formed endogenously in unexposed rats, mice and humans. We have developed ultrasensitive and highly specific assays for the DNA adducts of VC. These include immunoaffinity/^{32}P-postlabeling methods for 1,N^6-ethenodeoxyadenosine (EdA) and 3,N^4-ethenodeoxycytidine (EdC), and GC/MS methods for N^2,3-ethenoguanine (EG). By adding animals to the reproductive and developmental toxicity studies, we will be able to assess the effect of exposure concentration on 1) the molecular dose of etheno adducts, the primary promutagenic DNA adducts of VC; 2) determine if there are differences in the dose-response between adult and weanling rats; 3) examine the major DNA repair pathways for VC DNA adducts; and 4) characterize the utility of using DNA adducts excreted in the urine as a biomarker of exposure.

Molecular Dosimetry Studies in Nonparenchymal Cells (NPC) versus Hepatocytes

Understanding why VC targets the endothelial cell for its carcinogenic effect is critical for proper risk assessment. We have preliminary data on EG in vinyl fluoride-exposed rats (2500 ppm, 6 hr/day, 5 days/wk, 4 wks) which shows that the number of adducts is ~2.5-fold higher in NPC than hepatocytes, even though metabolism of VF to its electrophile is thought to only take place in hepatocytes. Since the NPC are exposed by diffusion of VC or VF electrophiles from neighboring hepatocytes and therefore have lower exposures, this suggested that NPC were deficient in DNA repair activity for EG. To test this hypothesis, we have developed a RT-PCR method for quantitating methylpurine-DNA glycosylase (MPG) in tissues and cells. Preliminary data have shown that NPC have <20% of the MPG activity present in hepatocytes. In addition, we have shown that NPC have 5-fold greater numbers of abasic sites in their DNA. Abasic sites are formed when MPG excises a damaged base, such as EG, EA and EC. These data suggest that NPC are deficient in a second step in the DNA repair of VC adducts. We think that the most likely candidate is a deficiency in AP endonuclease. The difference between hepatocyte and NPC abasic sites was seen in rats exposed to VF, but not in controls. The combination of two defects in DNA repair in the target cells for VC carcinogenesis provides a strong scientific basis for the induction of hemangiosarcomas by this important chemical. In view of the fact that the increase in abasic sites was only seen in highly exposed rats shows the need to understand the dose response relationship for this endpoint. By conducting 4 week exposures to 0, 10, 100, and 1000 ppm VC and measuring etheno adducts, MPG expression and abasic sites, we will be able to determine if nonlinearities exist in the molecular dose of VC, in MPG expression, and in the number of abasic sites present in the DNA. Our hypothesis is that EG will show a supralinear dose response; that MPG will be similar in exposed and unexposed hepatocytes, but lower in both exposed and unexposed NPC; but that abasic sites will exhibit a sublinear dose response characteristic of saturation of DNA repair in NPC, but not hepatocytes.