Studies Using [$^{13}C_2$]-VC

Our data showing endogenous formation of etheno DNA adducts that are identical to those induced by VC have important implications. We had previously published a paper that concluded that the DNA adducts of VC were highly persistent. We now believe that this was not correct, in that what we were calling persistent DNA adducts were actually the endogenously formed adducts at steady-state. By utilizing [$^{13}C_2$]-VC, the formation and repair of VC-induced DNA adducts can be studied relative to those adducts formed endogenously. I believe that these studies will critically impact on any risk assessment dealing with low exposures, such as might be associated with accidental releases that reach the fenceline.

We will examine the amount of [$^{13}C_2$]-VC induced EG in rats exposed to 10, 100, or 1000 ppm for 5 days (6 hrs/day). These same animals will be housed in metabolism cages for 3 days prior to exposure and during each day's post exposure holding period (days 1-4) so that urine can be collected. The urine will be analyzed for endogenous and induced DNA adducts that have been repaired by the MPG pathway using GC/MS. We will also develop immunoaffinity-LC/MS methods for all three etheno bases. In addition, it may be possible to identify and quantitate [$^{13}C_2$]-VC metabolites in the urine using NMR. A second set of [$^{13}C_2$]-VC exposures is contemplated that would consist of single exposures. These will be designed after we get data from the above study.

Significance

We have developed highly sensitive and specific assays for the promutagenic DNA adducts of vinyl chloride over the past 10 years. By exposing additional animals in the Huntingdon study, we can use these methods to answer several questions related to improved risk assessment of VC. First, the molecular dosimetry data will be the only information of its type that addresses dose-response. All other data have come from high exposures of 500-600 ppm. Second, we will address the issue of differences between weanling and adult animals. While we already know that preweanling rats are more sensitive than adults to the formation of DNA adducts and carcinogenesis at high concentrations, it is not known if this is true for low exposures. Since the only VC exposures that children encounter would be extremely low, the 10 ppm data are of particular importance. Just

because preweanling rats exposed to concentrations that saturate metabolism develop more adducts does not necessarily mean that young animals exposed to low concentrations will also develop more DNA adducts than adults. Our recent development of an assay for MPG and the finding that NPC are deficient in this DNA repair pathway provides the first mechanistic data that explains why VC causes hemangiosarcomas. The additional finding of greatly increased numbers of abasic sites in the NPC DNA further highlights the importance of cell-specific differences in DNA repair. The experiments outlined above will provide compelling data on the importance of DNA repair in VC carcinogenesis. Finally, the use of $[^{13}C_2]$-VC will allow us to differentiate between induced and endogenous DNA adduct formation. If large numbers of induced EG are formed, they may lead to increased numbers of endogenous adducts due to competition for DNA repair. On the other hand, we will be able to determine the extent of increased DNA damage relative to endogenous adducts for all three exposure groups. For example, we might find that 10 ppm VC for 5 days only doubles the amount of EG present endogenously. Such a finding should have great impact on low dose risk assessments. We would then look at various single exposures to simulate fenceline exposure scenarios to determine if there was any detectable increase in risk. Similar studies could be used to mimic occupational or environmental exposures to VC.

Protocols

Study I. Adult Rats Exposed to Vinyl Chloride for 1 Week

Route: Whole body exposure conducted during the two generation animal portion of the main study

Exposure groups: 0, 10, 100, or 1000 ppm

No. of rats/group: 32

Total: 128

Exposure duration: 1 week (6 hr/day, 5 days)

Species/strain: CD Rat

Age: Approximately 12 weeks of age

Sex: Males

Sacrifice: 8 rats/ group at end of last exposure (non-perfused), and 8 rats/ group at end of last exposure and at 3 and 7 days post-exposure (perfused)

Urine collections: during Days 1-4 of exposure (collected during nonexposure times)

Study II. Weanling Rats Exposed to Vinyl Chloride for 1 Week

Route: Whole body exposure conducted during the two generation animal portion of the main study

Exposure groups: 0, 10, 100, or 1000 ppm

No. of rats/group: 32

Total: 128

Exposure duration: 1 week (6 hr/day, 5 days/week)

Species/strain: CD Rat

Age: Approximately 21 days of age

Sex: Males

Sacrifice: 8 rats/ group at end of last exposure (non-perfused), and 8 rats/ group at end of last exposure and at 3 and 7 days post-exposure (perfused)

Urine collections: during Days 1-4 of exposure (collected during nonexposure times)

Study III. Adult Rats Exposed to Vinyl Chloride for 4 Weeks

Route: Whole body exposure conducted during the two generation animal portion of the main study

Exposure groups: 0, 10, 100, or 1000 ppm

No. of rats/group: 32

Total: 128

Exposure duration: 4 weeks (6 hr/day, 5 days/week)

Species/strain: CD Rat

Age: Approximately 12 weeks of age

Sex: Males

Sacrifice: 8 rats/ group at end of last exposure (non-perfused), and 8 rats/ group at end of last exposure and at 3 and 7 days post-exposure (perfused)

Urine collections: during last week of exposure (collected during nonexposure times)

Study IV. Adult Rats Exposed to [$^{13}C_2$]-Vinyl Chloride for 1 Week

Route: Nose only exposure

Exposure groups: 10, 100, or 1000 ppm

No. of rats/group: 8

Total: 24

Exposure duration: 5 days (6 hr/day)

Species/strain: CD Rat

Age: Approximately 12 weeks of age

Sex: Males

Sacrifice: 8 rats/ group at end of last exposure (perfused)

Urine collections: 3 days prior to exposure and Days 1-4 during exposure (collected during nonexposure times)