Radical Intermediates of Haloacetic Acids

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LAY SUMMARY OF AIR FORCE GRANT

The purpose of the study is to determine the mechanism by which a common environmental pollutant, 1,1,2-trichloroethylene, causes cancer. Trichloroethylene is a solvent that is widely used by the military and by industry to clean metal parts. The chemical also has applications in adhesives, paint stripping, dry cleaning, and the manufacture of pharmaceuticals and textiles. Hundreds of millions of pounds of trichloroethylene are produced each year. Because of its widespread use and often improper disposal, trichloroethylene has become a frequent environmental contaminant of ground and surface waters and is among the ten most cited chemicals at hazardous waste sites. Public exposure to trichloroethylene occurs by means of contaminated waters that ultimately find their way into the water used for human consumption. Trichloroethylene produces hepatocellular carcinomas and lung tumors in the mouse and renal tubular adenomas and testicular tumors in the rat. However, a role for trichloroethylene in human cancer has not been firmly established.

Past research has shown that trichloroethylene is metabolized in the liver to a variety of products that include chloral hydrate, dichloroacetic acid, trichloroacetic acid, and trichloroethanol. In particular, dichloroacetic acid and trichloroacetic acid ("haloacetic acids") are believed to be responsible for the carcinogenesis of trichloroethylene. Bernofsky hypothesizes that these haloacetic acids give rise to free radical intermediates that are genotoxic by virtue of their ability to form stable adducts with DNA. Radicals derived from the haloacetic acids would also be capable of damaging proteins and initiating the process of lipid peroxidation.

Free radicals produced in living cells are reactive, short-lived chemical species that contain an unpaired electron. Ordinarily, they cannot be directly observed. Their presence is often inferred by examination of target structures that have been damaged. Alternatively, free radicals can be trapped by reaction with certain nitrones or nitroso compounds called spin-trapping agents that can combine with the primary radical to yield a longer-lived radical derivative (spin adduct) that can be observed by electron paramagnetic resonance spectroscopy (EPR). In many instances, a radical can be identified by the characteristic EPR spectrum of its spin adduct. However, in living cells the detection of a free radical by spin trapping can be difficult if the spin adduct is itself metabolized to other products.

Bernofsky has proposed a unique new method to deal with the latter situation. Using mouse liver hepatocytes, he will examine the formation of free radicals indirectly, through the use of a ^{14}C-labeled spin-trapping agent to form a radioactive spin adduct that subsequently will be converted to a stable derivative and quantitated by means of high-pressure liquid chromatography (HPLC). The rationale for this approach is based on recent evidence showing that viable cells have the ability to metabolize spin adducts to EPR-silent species, which could lead to a significant underestimate of radicals that have been trapped.

In separate experiments, the nature of the DNA adducts that are formed will be determined by isolating the adducted DNA, digesting it with nucleases, and identifying the modified bases by means of HPLC and mass spectroscopy. Isolation of the modified bases will be facilitated by using ^{14}C-labeled haloacetic acids to form the adducted DNA. By analyzing the separated bases for the presence of radioactivity, it will be possible to determine the extent to which they have been modified by the haloacetic acid. The formation of DNA adducts from trichloroethylene and its metabolites would explain the carcinogenicity of these substances.