FORMATION OF 1,N⁶-ETHENODEOXYADENOSINE AND 3,N⁴-ETHENODEOXYCYTIDINE IN DNA FROM SEVERAL ORGANS OF RATS EXPOSED TO VINYL CHLORIDE

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ABSTRACT

Seven-day old (group I) and 28-day old (group II) BDVI rats were exposed for two weeks to 500 ppm vinyl chloride (VC) in air [7 hours per day, for 7 days (group I) or 5 days (group II) per week]. DNA from several organs was analysed for the formation of three VC adducts: 7-(2-oxoethyl)-guanine (oxetG), 1,N⁶-ethenodeoxyadenosine (εAdR) and 3,N⁴-ethenodeoxycytidine (εCdR). oxetG was measured as 7-(2-hydroxy-2-[³H]-ethyl)guanine by a post-labelling/HPLC procedure. εAdR and εCdR were dosed from enzymatic DNA hydrolysates separated by reversed-phase HPLC: a competitive radioimmunoassay (RIA) in the presence of specific murine monoclonal antibodies (Mab; obtained in collaboration with M.F. Rajewsky and G. Eberle) was used. Both ethenonucleosides were detected in the DNA from the liver, lung and brain of group I rats, at levels (fmoles/mg DNA) ranging from 62 to 133 for εAdR and 162 to 394 for εCdR. Molar ratios of oxetG/εCdR and oxetG/εAdR in DNA were about 30 and 80, respectively, in these three organs. In contrast, εAdR and εCdR were not detected (detection limit, 25 fmoles/mg DNA) in the kidney of group I nor in the liver from group II rats. These findings are discussed in relation to the organotropism of VC-induced carcinogenesis.

INTRODUCTION

VC has been implicated in the etiology of occupational cancers, in particular of hepatic angiosarcomas (Creech and Johnson, 1974; Forman, et al., 1985) and it is generally accepted that the carcinogenic and mutagenic effects of VC are due to its reactive metabolite, chloroethylene oxide (review articles: Bartsch and Montesano, 1975; Barbin and Bartsch, 1986; Bolt, 1986). Four adducts of VC (chloroethylene oxide) with nucleic acid bases are presently known (Figure 1). Following short treatments of rodents with [¹⁴C]-VC, oxetG was
the major adduct observed in DNA (Osterman-Golkar, et al., 1977; Laib, et al., 1981), and low amounts of N\textsuperscript{2}, 3-ethenoguanine (\textepsilon G) were detected in rat liver DNA (Laib, 1986). Under similar experimental conditions, \textsuperscript{[\textsuperscript{14}C]}-labelled 1,N\textsuperscript{6}-ethenoadenine and 3,N\textsuperscript{4}-ethenocytosine moieties were easily detected in RNA but not in DNA of rat liver (Laib, et al., 1981). In contrast, Green and Hathway (1978) presented limited mass-spectral evidence, but no quantitative data, for the formation of \textepsilon CdR and, tentatively, \textepsilon AdR in liver DNA of rats after a two-year exposure to VC. Recently, a sensitive immunoanalytical method using Mab was developed for dosing \textepsilon AdR and \textepsilon CdR (Eberle, et al., 1989). With this method, Eberle, et al., could demonstrate the formation of, and quantitate both ethenoadducts in lung and liver DNA from infant Sprague-Dawley rats exposed to VC.

The detection of ethenobases in DNA may be an important step towards the understanding of VC-induced mutagenesis and carcinogenesis. Indeed, \textit{in vitro} replication or transcription fidelity assays using synthetic templates suggest that the three ethenobases, but not oxetG may be potential promutagenic lesions of VC (Barbin and Bartsch, 1986; Singer, et al., 1987; Bolt, 1988). Therefore, determination of the kinetics of formation/repair of ethenobases \textit{in vivo} should permit to further elucidate how VC exerts its genotoxic effects. As a first step towards this aim, we exposed young BDVI rats to VC and analysed the formation of \textepsilon AdR and \textepsilon CdR in the DNA of several organs known to be sensitive to VC-induced carcinogenesis. In addition, we determined the levels of oxetG.

\textbf{Figure 1.} Vinyl chloride metabolites and nucleic acid adducts.