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Review articles

Analysis of macromolecular ethylene oxide adducts

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Summary. Ethylene oxide is a carcinogenic compound which is also an ethylene metabolite. Ethylene oxide forms macromulecular adducts with proteins and nucleic acids. Targets in proteins are the amino acids cysteine, histidine and valine (if N-terminal, as in hemoglobin). The major DNA adduct is 7-(2-hydroxyethyl)-guanine. Methods for detection of this adduct include radiolabelling and GC-MS. The sensitivity of current GC/MS methods can be improved by selective enrichment of adducts from DNA samples. Studies in this direction are presently being performed.

Key words: Ethylene – Ethylene oxide – Protein adducts – DNA adducts

Introduction

The experimental induction of systemic malignant tumors in rats after inhalation of ethylene oxide (Snellings et al. 1984; Lynch et al. 1984) has promoted research on biomonitoring strategies for individuals exposed to this compound.

Ethylene can be converted in the organism into ethylene oxide (Filser and Bolt 1983). Although a long-term inhalation carcinogenicity study in rats with ethylene was negative (Hamm et al. 1984), a risk of ethylene exposure, mediated by transformation to ethylene oxide, has been inferred (Osterman-Golkar and Ehrenberg 1982). To quantitate this risk, methods of analysis of macromolecular adducts induced by exposure to ethylene and ethylene oxide (Ehrenberg et al. 1977; Segerbäck 1983) and pharmacokinetic methods (Bolt et al. 1984; Filser and Bolt 1984; Bolt and Filser 1984) have been used. Based on this background, a comparison of different methodologies, by which macromolecular adducts of ethylene oxide can be assessed, is needed.

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Results of different methods

Alkylation of proteins

Figure 1 shows the tyes of macromolecular adducts of ethylene oxide reported to date. In proteins, proven targets (Segerbäck 1983) are cysteine, histidine and valine (if N-terminal, as in hemoglobin). After experimental exposure to ethylene oxide and ethylene in vivo, and after reaction of hemoglobin in vitro with ethylene oxide, the highest degree of alkylation is found at the SH-group of cysteine, followed by N-terminal valine alkylation and by the two isomeric alkylation products at the imidazole-ring nitrogen atoms of histidine (Segerbäck 1983).

Recently, determination of the degree of alkylation of N-terminal valine in hemoglobin has been facilitated by the introduction of a modified Edman degradation technique (Törnqvist et al. 1986a). This degredation is followed by a GC/MS determination of the adduct. Results obtained with human samples obtained from exposed ethylene oxide workers demonstrated the general applicability of this analytical method. Comparisons of alkylation (hydroxyethylation) of histidine and N-terminal valine showed an excellent correlation of both parameters in differently assessed individuals, however, higher levels of background alkylation were recorded for hydroxyethyl-histidine. Even in cases where the hydroxy-

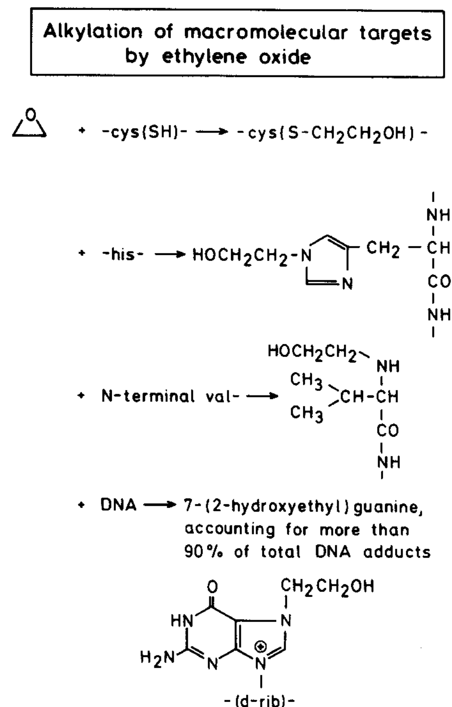

Fig. 1. Alkylation sites for ethylene oxide in proteins and DNA

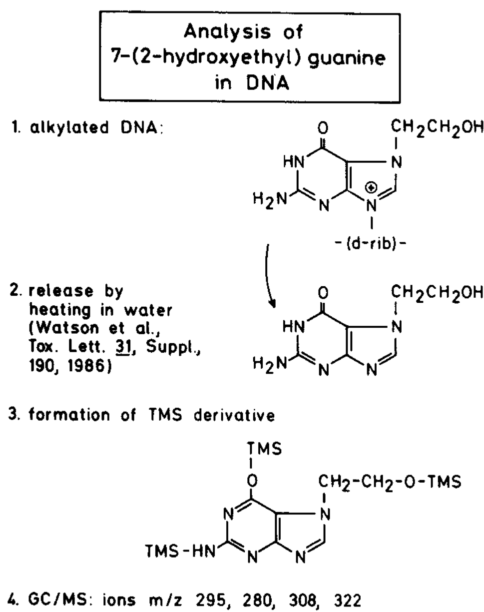

Fig. 2. Technical procedure for analysis of the major ethylene oxide/DNA adduct

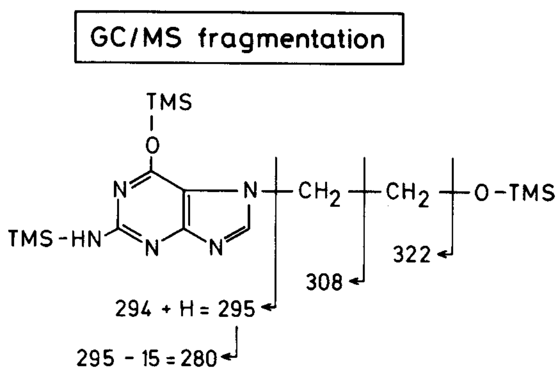

Fig. 3. Fragmentation pattern of the TMS-derivative of the major ethylene oxide/DNA adduct

ethyl-valine content in hemoglobin approximated zero, there was ca 0.6 nmol hydroxyethyl-histidine per g hemoglobin remaining (Farmer et al. 1986). Although a first risk assessment for ethylene as inhaled during cigarette smoking has been published based on valine alkylation in hemoglobin (Törnqvist et al. 1986b), the discrepancy in alkylation of different targets in the protein makes it difficult to arrive at risk conclusions based on protein alkylation data only, expecially at the low exposure levels. For this purpose a validation by a quantitation of DNA adducts would be needed.

Alkylation of DNA

The main product of reaction of ethylene oxide with DNA (see Fig. 1) is 7-(2-hydroxyethyl)-guanine (Segerbäck 1983). In vitro, 7-(2-hydroxyethyl)-guanosine has been prepared by Brookes and Lawley (1961) *via* reaction of guanosine with ethylene oxide. This principal reaction of ethylene oxide was later confirmed by Ashby et al. (1982). The preference for 7-alkylation of guanine is explained by the electron density distribution of purines (Hurst 1980). Hence, 7-alkylated guanine may be used as an (abundant) indicator lesion in DNA which has been in contact with ethylene oxide.

A working schedule is shown in Fig. 2. A selective enrichment of 7-alkylated purines present in carcinogen-modified DNA is possible by releasing them upon heating the DNA (Watson et al. 1986). After formation of the TMS-derivative, the selective ions of the adduct can be monitored by GC/MS.

Figure 3 shows the fragmentation pattern of the ethylene oxide-DNA-TMS derivate, leading to the ions listed in Fig. 2 (Föst 1987).

Assay sensitivity (DNA adducts)

The sensitivity of a method to detect DNA adducts is compromised by the absolute detection limit and the amount of material required for analysis.

Table 1 compares available methodologies for the major ethylene oxide adduct, 7-(hydroxyethyl)-guanine, with those reported for other related adducts.

Table 1. Sensitivity of assays for the detection of carcinogen-DNA adducts

Method	Limit of detection (fmol)	Amount of DNA (µg)	Modified nucleotide detected per nucleotides in DNA	Reference
HPLC, $UV_{254\,nm}$	100000	3500	$1:1\times10^5$	Jeffrey (1986)
Fluorescence	30	100	$1:1\times10^7$	Jeffrey (1986)
RIA, O^6-EtdG	40	10000	$1:8\times10^8$	Jeffrey (1986)
Fluorescence labelled antibodies, O^6-EtdG	1×10^{-6}	6×10^{-6}	$1:2\times10^7$	Adamkiewicz et al. (1983)
^{14}C labelled carcinogen (ethylene oxide, 65 Ci/mol)	280	5000	$1:8\times10^7$	Potter (1986)
^3H labelled carcinogen	1.5	5000	$1:1\times10^{10}$	Jeffrey (1985)
^{32}P-postlabelling (5mdC)	50	1	$1:6\times10^4$	Wilson et al. (1986)
GC/MS:				
5mdC	13	1	$1:2\times10^5$	Randt (1986)
6-hydroxy-guanine	10	1	$1:3\times10^5$	Dizdaroglu and Bergtold (1986)
7-(2-hydroxyethyl)-guanine	100	1	$1:3\times10^4$	Föst (1987)
7-(2-hydroxyprop-1-yl)-guanine	100	1	$1:3\times10^4$	Föst (1987)
7-methyl-guanine	100	1	$1:3\times10^4$	Föst (1987)
GC-NICI-MS/GC-ECD, O^4-EtdT	0.2	1	$1:2\times10^7$	Giese et al. (1985)

Without selective enrichment of adducts out of the DNA or DNA hydrolysate, an assay should indicate at least one specific lesion out of 100000 nucleosides. This is, in principle, met by fluorescence, immunological and experimental radioactivity labelling techniques. As radioactive labelling cannot be used for purposes of human exposure monitoring, interest is focussed on immunological determinations and a further development of GC/MS techniques. One aspect of improving sensitivity may be the use of electrophore labelling (Giese et al. 1985), but selective enrichment of adducts (as shown in Fig. 2) may also be a way to overcome the present difficulties.

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