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Guest editorial

Experimental toxicology of formaldehyde *

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Summary. Formaldehyde is a reactive chemical which undergoes spontaneous reactions with various cellular constituents. Mutagenicity data may be interpreted on the background of this behavior. Mice are better able to reduce the irritating effect of formaldehyde than rats and to reduce their ventilation rate when formaldehyde acts on the respiratory tract. Subacute exposure of rats to concentrations higher than 2 ppm inhibits mucociliary clearance of the nasal epithelium and leads to progressive histological and ultrastructural lesions at this site. The occurrence of squamous cell carcinomas of the nasal epithelium of rats after 2 years inhalation of 14.3 ppm formaldehyde (CIIT study) is probably the result of chronic and recurrent local toxicity; this is supported by species differences in susceptibility to the tissue damaging and carcinogenic effect of formaldehyde (rat, mouse, hamster). Data on formaldehyde-DNA interaction further support the argument that a direct risk extrapolation from the formaldehyde effects in rats to those expected for man is not possible.

Key words: Formaldehyde – Mutagenicity – Metabolism – Biochemistry – Carcinogenicity

Introduction

Toxicological effects of formaldehyde have been widely discussed during the last few years. The primary focus was on the pathogenesis and interpretation of nasal tumours found in long-term rodent bioassays. The present review has ben set up to summarize the main arguments on which the regulatory discussion has focused.

Biochemistry

One of the main reasons for the use of formaldehyde is its antimicrobial activity. This is intrinsically linked with its reactivity with functional groups of macromolecules, and also explains its damaging effect on cells and tissues, its irritating effects, allergenic properties, and mutagenicity (Fig. 1). These different effects are therefore interconnected.

Formaldehyde is known to react non enzymatically with amino and sulfhydryl groups. For instance, it forms thiazolidine-4-carboxylic acid with cysteine and hydroxymethyl adducts with urea; these adducts are found in urine of formaldehyde-treated animals (Mashford and Jones 1982). With proteins, in a first step, reversible adducts are formed (Fig. 1). Hence, a major portion of formaldehyde in tissues is present in "bound" form.

Of major importance is the (reversible) addition to glutathione (GSH). Glutathione is the cofactor of formaldehyde dehydrogenase; in fact, the real substrate of the enzyme is the adduct S-hydroxymethylglutathione which is oxidized (further cofactor: NAD) to formylglutathione. The latter (reversibly) dissociates to GSH and formic acid (Casanova-Schmitz et al. 1984a).

Hence, metabolism and toxicity of formaldehyde in cells (as demonstrated by isolated rat hepatocytes) are dependent upon the intracellular concentration of GSH. For instance, pretreatment with diethyl maleate (which depletes GSH) decreases the rate of disappearance of formaldehyde and thereby potentiates formaldehyde toxicity. It has been hypothesized that formaldehyde toxicity which can be visualized in GSH-

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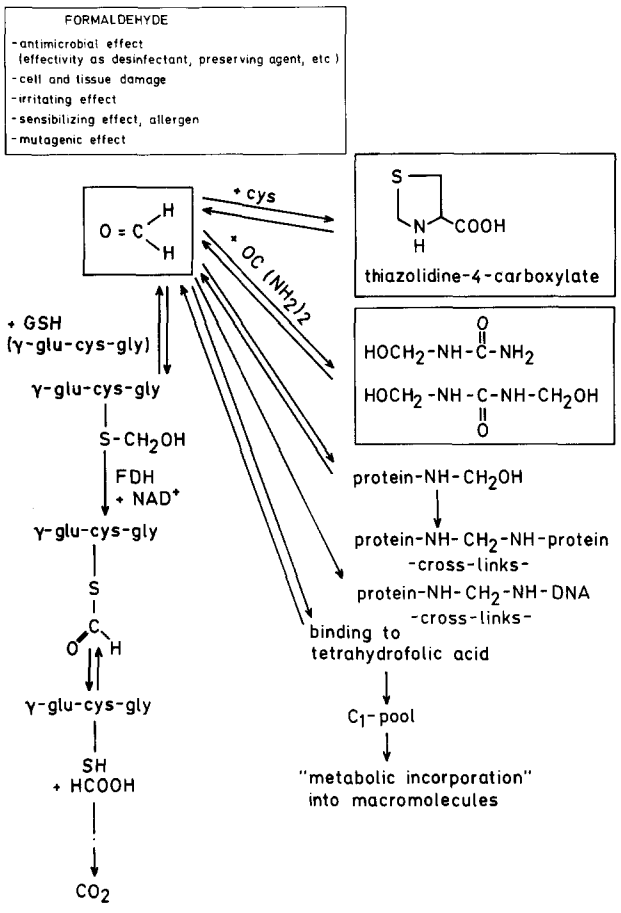

Fig. 1. Biological reactions and metabolism of formaldehyde

depleted hepatocytes may be mediated by free radicals as a result of the depletion of a critical cellular pool of GSH (Ku and Billings 1984).

It should also be noted that acrolein which depletes GSH in rat nasal mucosa, but does not cause DNA-protein cross-links, leads to an amplification of these typical formaldehyde lesions if it acts on this target together with formaldehyde (Lam et al. 1985).

For the industrial chemical methyl chloride, formaldehyde has been discussed as a metabolically formed intermediate (Bus 1982), and carcinogenic effects of high doses of methyl chloride on kidneys of male rats have been ascribed to this metabolite (DFG 1984). However, we have found that even high (1.000 ppm) and repeated exposure to methyl chloride did not elevate tissue formaldehyde levels in liver or kidneys of rats or mice. Also, DNA characteristics of formaldehyde (i.e. DNA-protein cross-links) were not detected under these conditions (unpublished data). This confirms the view that formaldehyde exerts carcinogenic effects only locally. This view is in full agreement with recent data of Lutz (1987) who found that even large doses of precursors of endogeneous formaldehyde (methanol, aminopyrine) do not lead to any significant increase in hepatic DNA-protein cross-links.

After binding to tetrahydrofolic acid (Fig. 1) formaldehyde molecules enter the C_1 pool of intermediary metabolism. Because the attachment to tetrahydrofolic acid is a reversible reaction, there is a physiological presence of formaldehyde in all cells. The phyiological concentration of formaldehyde in human blood has been reported to be 2.6 µg/g (Heck and Casanova-Schmitz 1984). Inhalation of low levels of formaldehyde (1.9 ppm for 40 min) did not elevate this. The incorporation of ^{14}C-labeled formaldehyde into the C_1 pool leads to "metabolic incorporation" into macromolecules, e.g., into nucleic acids. This must be well differentiated from DNA alkylation (Casanova-Schmitz et al. 1984b).

Reaction of formaldehyde with amino groups leads, in a first step, to formation of (unstable) hydroxymethyl (methylol) adducts. In a second (slow) reaction a second amino group may be bound under formation of a methylene cross-linking bridge. Such cross-links are most probably responsible for the protein-denaturing properties of formaldehyde and for its cytotoxicity.

The reactivity of formaldehyde with amino groups of nucleic acids is most probably linked to the genetic effects of formaldehyde. Such lesions were noted with RNA and single-stranded DNA, but not with double-stranded DNA. Exocylic amino groups of purines seem to be especially susceptible (Singer and Kusmierek 1982).

Mutagenicity

The genetic toxicology of formaldehyde has been recently reviewed (ECETOC 1982; Swenberg et al. 1983a; Ulsamer et al. 1984). In general, the data available show that formaldehyde is mutagenic in different test systems, especially when high concentrations act directly on cells (gene and chromosome mutations). Also, positive cell transformation assays have been reported in vitro. After inhalation of the compound local DNA adducts were observed in rats without simultaneous systemic genetic effects (Casanova-Schmitz et al. 1984b).

Recently, experiments on induction of sister chromatid exchanges in human lymphocyte cultures (Kreiger and Garry 1983) have demonstrated no significant sister chromatid exchange response below an apparent "threshold" of 5 µg/ml culture medium. In accordance with this, the cytotoxic dose-response curve showed a biphasic pattern with a marked increase of slope at about 10 µg formalgehyde/ml. Recent studies on UDS (unscheduled DNA synthesis) in primary cultures of human bronchial epithelial cells (Doolittle et al. 1985) have demonstrated no response to formaldehyde in this system. An earlier finding that formaldehyde may inhibit DNA repair (Grafstrom et al. 1983) has not been confirmed (Snyder and Van Houten 1986).

In general, the observed mutagenic effects of formaldehyde are probably due to covalent interaction with DNA bases. Formaldehyde damage induced in DNA of different human cell culture systems comprises DNA-protein cross-links and DNA single-strand breaks; these lesions undergo efficient repair by complex mechanisms (Grafstrom et al. 1984).

Acute and subacute effects

Acute doses of formaldehyde cause sensory irritation. High concentrations (over 100 ppm) cause salivation, dyspnea, convulsions, and death (NIOSH 1980). Experimentally, the RD_{50} (concentration causing a respiratory rate depression by 50%) was 32 ppm in rats, but 4.9 ppm in mice. Mice are therefore better able to reduce the irritating effect of formaldehyde; they are able to minimize inhalation of irritating concentrations of the compound more than rats (Chang et al. 1981). Tolerance to sensory irritation was observed in rats exposed to 28 ppm for 4 days, but not in those exposed to 15 ppm for 1, 4, or 10 days (Chang and Barrow 1984). A comparison with other aldehydes showed that formaldehyde was nearly 1000 times more potent than saturated aliphatic aldehydes with two or morge carbon atoms (Steinhagen and Barrow 1984). Recent sensitization experiments in guinea pigs (Lee et al. 1984) have shown that formaldehyde acts in this species as a skin sensitizer without causing detectable respiratory hypersensitivity.

Subacute inhalation studies in rats, 6 h/day, for periods between 1 day and 3 weeks, with exposure concentrations of 0, 0.5, 2, 6, or 15 ppm formaldehyde showed severe impairment of the nasal mucociliary clearance at 15 ppm, becoming progressively more extensive during repeated exposures for up to 2 weeks. Inhibition of mucociliary clearance was less severe at 6 ppm, minimal at 2 ppm, and not detectable at 0.5 ppm exposure. The distribution of epithelial lesions, histologically identified, correlated with the distribution of defective mucociliary function; the latter was regarded as a very sensitive indicator of toxicity (Morgan et al. 1986a).

When rats were exposed for 1–4 days to different concentrations of formaldehyde (0.5, 2, 6, 15 ppm; 6 h/day) ultrastructural changes of the nasal respiratory epithelium in the lower exposure groups (up to 2 ppm) were very slight only. Exposure to 6 or 15 ppm, however, caused severe and dose-related respiratory epithelial injury (Monteiro-Riviere and Popp 1986).

Carcinogenicity

The "CIIT study" (Swenberg et al. 1980; Kerns et al. 1983; Swenberg et al. 1983; Morgan et al. 1986b), on which recent discussions have focused, was performed on male and female F-344 rats and $B_6C_3F_1$ mice exposed to 2.0, 5.6 or 14.3 ppm formaldehyde for 6 h/day and 5 days/week over 24 months. Of main concern was the induction of squamous cell carcinomas in the nasal passages, occuring primarily in rats exposed at the highest concentration. A second lesion of concern was polypoid adenomas, observed in treated and control rats. The results in rats were also basically confirmed by a second study (Albert et al. 1982; Sellakumar et al. 1985) with Sprague-Dawley rats exposed to 14 ppm formaldehyde.

In the "CIIT study" mice showed no morphological changes at 2 ppm, whereas 5.6 and especially 14.3 ppm led to rhinitis, and to dysplasia and metaplasia of the nasal epithelium. Two (male) mice of the highest dose group developed squamous cell carcinoma (not statistically significant). The histological changes of dysplasia and metaplasia were more marked in rats. Changes were also observed at 2 ppm in this species. At 5.6 ppm one male and one female rat, but at 14.3 ppm nearly half of all rats developed squamous cell carcinomas. At the highest dose dysplasia and metaplasia of the trachea were also noticed. It has also been demonstrated that basing tumor data on the actually delivered dose (which is species-dependent because of differences in respiration physiologiy) has a substantial impact on the outcome of mathematical risk assessments (Starr and Buck 1984).

Formal criticism of the study has been raised over its general outline (BMJFG 1984). In the rat study, the upper two doses involved severe toxic and lethal effects (20% mortality at 18 months on 14.3 ppm). Severe rhinitis often led to dysplasia and subsequent death. It has been argued that low doses ought to have been selected. A study in hamsters (Dalbey 1982) with 10 ppm formaldehyde was negative in terms of tumor formation.

Formaldehyde-DNA interaction

Data on formaldehyde-DNA interaction have been utilized for interpretation of the biossay data (Casanova-Schmitz et al. 1984b) and have been controversely discussed (Cohn et al. 1985).

The hydroxymethyl adducts of formaldehyde with DNA (e.g., N^6-hydroxymethyl-adenine) are highly unstable (Feldmann 1973; Lukashin et al. 1976) whereas methylene bridges in formaldehyde-treated nucleohistones are regarded as essentially irreversible (Brutlag et al. 1969).

By contrast, no evidence for formation by formaldehyde of DNA-DNA cross-links has been obtained experimentally with mammalian cells (Ross and Shipley 1980; Bedford and Fox 1981; Ross et al. 1981; Har-

ris et al. 1983), and steric considerations have rendered this possibility unlikely (Bedford and Fox 1981). With this background the formation of DNA-protein cross-links which occurs in rodent nasal respiratory mucosa (Casanova-Schmitz and Heck 1983) is regarded as an important lesion in the pathogenesis of formaldehyde-induced tumors (Lam et al. 1986). It is noteworthy that remarkable differences between different nucleoproteins exist in terms of "cross-linkable" DNA-protein contacts (Solomon and Varshavsky 1985).

In this connection, the general pattern of cell turnover has been used for further investigation. Formaldehyde exposure leads to an increase in the rate of cell turnover in the respiratory mucosa (which is the tissue from where the nasal squamous cell carcinomas originate). This increase is nonlinear with formaldehyde concentration, being undetectable at 2 ppm, highly significant at 6 ppm, then decreasing at 15 ppm due to cytotoxicity (Swenberg et al. 1983b). Such increased cell replication would substantially increase the number of (non double-stranded DNA) sites available for reaction with formaldehyde. This anticipation has been proven experimentally; the covalent binding of labeled formaldehyde to DNA of respiratory rat mucosa was not linear with the exposure concentration, but increased steeply between 2 and 6 ppm (Casanova-Schmitz et al. 1984b). This has been put forward as an important argument against the applicability of the usual risk estimates for formaldehyde.

Conclusions on risk extrapolation

On an international level, discussions of possible human risk on the basis of the animal experiments have been controversial (IARC 1982; BMFT 1984; Federal Register 1985). The high incidence of experimental tumors in rats at 14.3 ppm, together with the mutagenic properties and the possibility of DNA interaction have been the main arguments in favor of a substantial human carcinogenic risk which ought to be calculated by conventional risk extrapolation models.

On the other hand, the following arguments have been used against a direct risk extrapolation to humans (BMJFG 1984):

- species differences in target tissue doses due to differences in respiration physiology;
- the highest dose in the CIIT rat experiment producing excessive toxicity and mortality;
- local cell and tissue lesions as being necessary precursor stages before tumors develop;
- dose response of cell proliferation leading to higher proportions of single-stranded DNA which is susceptible to the generation of DNA-protein cross-links;
- non linear dose-response of covalent formaldehyde-DNA interaction;
- endogenous formation of formaldehyde and physiological formaldehyde levels;
- rapid detoxication of formaldehyde, if present in doses which are not excessive;
- formaldehyde seems not to act systemically.

The recommendations of various official bodies, on national and international levels, mostly considered such arguments. The concept that humans very probably are less susceptible than test rodents, especially rats, has widely been accepted (Squire and Cameron 1984).

However, it appears that still more basic research is needed to clarify the situation. Several lines of evidence (as discussed above) suggest that a minor genotoxicity of formaldehyde is expressed only at higher dose levels where increased cytotoxicity and regenerative cell division are found (Lutz 1986a). Hence, in addition to the data of Casanova-Schmitz et al. (1984b), more information on the dose-response of DNA-protein cross-links, possibly by using alkaline elution techniques, is required.

The question of formation and dose-response of DNA strand breaks (Grafstrom et al. 1984) needs clarification. Studies on interaction in vivo of formaldehyde with other irritating agents (Lam et al. 1985; Sellakumar et al. 1985) should include wider ranges of irritant doses to learn more about dose-response relationships.

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