[CANCER RESEARCH 46, 5682–5686, November 1986]

Formation of Cyclic Adducts of Deoxyguanosine with the Aldehydes *trans*-4-Hydroxy-2-hexenal and *trans*-4-Hydroxy-2-nonenal *in Vitro*[1]

Carl K. Winter, H. J. Segall,[2] and William F. Haddon

Department of Veterinary Pharmacology and Toxicology, University of California, Davis, California 95616 [C. K. W., H. J. S.], and Western Regional Research Center, United States Department of Agriculture, Berkeley, California 94710 [W. F. H.]

ABSTRACT

trans-4-Hydroxy-2-hexenal (t-4HH), a reactive metabolite isolated from the pyrrolizidine alkaloid senecionine, and *trans*-4-hydroxy-2-nonenal (t-4HN), a product of lipid peroxidation, reacted nonenzymatically with deoxyguanosine at pH 7.4 at 37°C *in vitro* with each compound yielding two pairs of diastereomeric adducts. Adducts were isolated using reverse phase high-performance liquid chromatography and were characterized by their mass spectra and proton magnetic resonance spectra. Adducts 1 and 2 from t-4HH were assigned the structures 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-8*R*-hydroxy-6*S*[1-(*R* and *S*)hydroxypropyl]pyramido[1,2-*a*]purine-10-(3*H*)one and Adducts 3 and 4 were assigned the structures 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-8*S*-hydroxy-6*R*-[1-(*R* and *S*)hydroxypropyl]pyramido[1,2-*a*]purine-10-(3*H*)one. Similar 6-hydroxyhexyl adducts were isolated in the reaction of deoxyguanosine with t-4HN. The reactions appear to involve Michael additions of the N^2 amino group of deoxyguanosine followed by cyclization at the 1-N site. This reaction mechanism is similar to that reported for deoxyguanosine adduct formation with the nonhydroxylated α,β-unsaturated aldehydes crotonaldehyde and acrolein. Total adduct formations following 16-h incubations were 0.91% for t-4HH and 0.85% for t-4HN. These results demonstrate that t-4HH and t-4HN possess the ability to alkylate deoxyguanosine *in vitro* and suggest possible mechanisms for 4-hydroxyalkenal and pyrrolizidine alkaloid genotoxicity.

INTRODUCTION

The 4-hydroxyalkenals represent an important family of compounds capable of producing a variety of biological effects. Members of the family have been shown to be mutagenic (1), cytotoxic (2–4), and carcinostatic (5–8). These latter two effects have been attributed to the ability of 4-hydroxyalkenals to react rapidly with low molecular weight thiol compounds (5).

It has been demonstrated that 4-hydroxyalkenals are produced during stimulated (ADP-Fe^{2+} or NADPH-Fe^{2+}) microsomal lipid peroxidation and that these compounds could be responsible for part of the destructive effects on cells and cell constituents caused by lipid peroxidation (9, 10). The major 4-hydroxyalkenal produced through lipid peroxidation is t-4HN[3] (Fig. 1) (9), while *trans*-4-hydroxy-2-octenal (9), *trans*-4-hydroxy-2-decenal (9), *trans*-4-hydroxy-2-undecenal (9), and *trans*-4,5-dihydroxy-2-decenal (11) have been produced in lesser amounts.

t-4HH (Fig. 1) was recently identified in our laboratory as a microsomal metabolite of the toxic macrocyclic pyrrolizidine alkaloid senecionine (12). The *in vivo* hepatic pathology caused by t-4HH appeared to be similar to that for senecionine, leading to the conclusion that t-4HH may play a major role in pyrrolizidine alkaloid toxicity.

Pyrrolizidine alkaloids are produced by a variety of plant species worldwide and are responsible for numerous livestock losses and human poisonings (13). Many pyrrolizidine alkaloids are hepatotoxins (14) and several have been shown to be carcinogenic (15) and genotoxic (16, 17). The toxic effects of these alkaloids has been previously attributed to the production of pyrrolic metabolites generated by the action of the hepatic microsomal enzyme system (18). These pyrroles are thought to produce their toxic effects by alkylating biological nucleophiles (19). Robertson (20) has demonstrated that one such pyrrole, dehydroretronecine, reacted *in vitro* with deoxyguanosine at physiological pH at the N^2 position to form a pair of covalently bound adducts. Binding of dehydroretronecine with other nucleosides has since been reported (21).

Acrolein and crotonaldehyde, both α,β-unsaturated aldehydes with structures similar to those of the 4-hydroxyalkenals, have been shown to alkylate deoxyguanosine *in vitro* by forming cyclic 1,N^2 adducts (22–24). This interaction may be highly significant as acrolein and crotonaldehyde demonstrated mutagenicity in *Salmonella typhimurium* in the absence of metabolic activation (25, 26). Crotonaldehyde has also recently been shown to be carcinogenic in F344 rats (27).

This study was performed to determine if the 4-hydroxyalkenals t-4HH and t-4HN were capable of forming similar deoxyguanosine adducts under physiological conditions. A demonstration of such covalent interaction could provide a possible explanation for the mutagenicity of 4-hydroxyalkenals. In addition, results could suggest an additional mechanism by which the pyrrolizidine alkaloids might ultimately damage genetic material.

MATERIALS AND METHODS

HPLC Analysis. The HPLC system consisted of two model 510 pumps with a model 680 solvent programmer and a model U6K injector (Waters Associates, Milford, MA). UV absorbance was measured at 254 nm with a model SF769Z detector (Kratos Analytical Instruments, Westwood, NJ) and recorded on a model 300 recorder (Linear Instruments, Irvine, CA). The following solvent elution systems were used: System 1, A 7.9-mm x 30-cm semipreparative ASI C_{18} reverse-phase column (Analytical Sciences, Inc., Santa Clara, CA) run isocratically at 2.0 ml/min with water:methanol (83:17, v/v); System 2, a semipreparative ASI C_{18} column run isocratically at 2.0 ml/min with water:methanol (65:35, v/v); System 3, a semipreparative ASI C_{18} column run isocratically at 2.0 ml/min with water:acetonitrile (95:5, v/v); System 4, a semipreparative ASI C_{18} column run isocratically at 2.0 ml/min with water:methanol (70:30, v/v); System 5, A 7.9-mm x 30-cm semipreparative Dupont Zorbax CN column (Analytical Sciences, Inc.) run isocratically at 2.25 ml/min with water:methanol (90:10, v/v).

360-MHz ^{1}H-NMR Spectra. Spectra were recorded at ambient temperatures (23–25°C) and at 40°C with a NMC-360 spectrometer interfaced with an NMC-1280 data system (Nicolet Magnetics, Fremont, CA). Dimethyl sulfoxide-d_6 (Aldrich Chemical Co., Milwaukee, WI)

Received 5/8/86; revised 7/29/86; accepted 7/30/86.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This study was supported in part by NIH Grant ES-03343; a Livestock Disease Research Laboratories Grant, School of Veterinary Medicine; and an NMR Faculty Grant, University of California. Presented in part at the 25th Annual Meeting of the Society of Toxicology, New Orleans, LA, March 1986 (Abstract No. 912).

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: t-4HN, *trans*-4-hydroxy-2-nonenal; t-4HH, *trans*-4-hydroxy-2-hexenal; HPLC, high performance liquid chromatography; NMR, nuclear magnetic resonance; FAB, fast atom bombardment; MS/MS, tandem mass spectrometry.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/46/11/5682/2425429/cr0460115682.pdf by guest on 01 December 2023

was used as the solvent while exchangeable protons were identified using deuterium oxide (Aldrich). All chemical shifts were referred to tetramethylsilane (Aldrich) = 0 ppm.

Mass Spectra. FAB mass spectra were obtained by adding 100–750 ng of adducts dissolved in methanol to about 2 µl of glycerol on a copper probe, followed by bombardment with 6-kV cesium ions on a VG 70/70HS mass spectrometer (VG-Organic, Ltd., Manchester, England). The cesium ion gun was constructed by Antek Corp. (Palo Alto, CA) and is similar to that described by Aberth *et al.* (28). MS/MS experiments used a ZAB-4F tandem double focusing mass spectrometer with BEEB geometry (VG-Organic, Ltd.).

Reaction of t-4HH and Deoxyguanosine. The synthesis of t-4HH was performed by the procedure of Erickson (29). *trans*-4-Hydroxy-2-hexenal (5.7 mg, 0.10 mmol) was combined with 5.3 mg (0.02 mmol) of deoxyguanosine (Sigma Chemical Co., St. Louis, MO) in 1 ml of 25 mM phosphate buffer, pH 7.4. The solution was incubated with constant stirring at 37°C for 16 h. Upon cooling, the mixture was filtered through 0.45-µm Acro LC13 filters (Gelman Sciences, Ann Arbor, MI) and separated by HPLC using System 1. Adducts 3 and 4 were isolated using this system; Adducts 1 and 2 were pooled and separated using System 3. Final purification of the four adducts was performed with System 5. Total adduct formation from this reaction was 0.91%.

To enable collection of sufficient material for NMR analysis, a larger scale reaction with 145 mg of t-4HH (1.27 mmol) and 139 mg of deoxyguanosine (0.52 mmol) was performed for 192 h at 37°C. The reaction was done in 25 ml of 25 mM carbonate buffer, pH 9.5, since Michael additions of amino groups to α,β-unsaturated aldehydes appear to proceed at higher rates with increased pH (30). Adducts were separated as previously described.

Reaction of t-4HN and Deoxyguanosine. The Erickson synthesis of t-4HH was modified by substituting hexaldehyde for propionaldehyde to produce t-4HN (29). An incubation with 7.3 mg (0.10 mmol) of t-4HN and 5.3 mg (0.02 mmol) of deoxyguanosine was performed for 16 h at 37°C in 1 ml of 25 mM phosphate buffer, pH 7.4. The mixtures were filtered as described previously and four major adducts were observed following HPLC using System 2. Adducts 3 and 4 of this mixture were isolated; no attempt was made to separate t-4HN-deoxyguanosine Adducts 1 and 2. Total adduct formation for this reaction was 0.85%.

A larger scale incubation was performed using 0.22 mmol of t-4HN and 0.08 mmol of deoxyguanosine in 3.9 ml of 25 mM carbonate buffer, pH 9.5. This reaction was done at 37°C for 192 h, and adducts were separated as described for the smaller scale reaction.

Reaction of *trans*-2-Hexenal and Deoxyguanosine. *trans*-2-Hexenal (98 mg, 1 mmol; Aldrich) was incubated with 53 mg (0.2 mmol) of deoxyguanosine in 20 ml of 25 mM phosphate buffer, pH 7.4, for 70 h at 37°C. Adducts were separated using HPLC System 4.

RESULTS

***trans*-4-Hydroxy-2-hexenal-Deoxyguanosine Adducts.** The HPLC chromatogram of the small scale t-4HH-deoxyguanosine reaction mixture using System 1 is shown in Fig. 2. Four major adducts absorbing at 254 nm were observed. No additional products were noted in the preparative reaction mixture.

Fig. 3A illustrates the positive ion FAB mass spectrum for t-4HH-deoxyguanosine Adduct 4. Peaks at *m/z* 382 (MH^+, measured mass 382.1704; $C_{16}H_{24}O_6N_5$ requires 382.1726, −5.8 ppm), and 404 (MNa^+) confirm the addition of a single molecule of t-4HH to deoxyguanosine. The negative ion FAB spectrum (Fig. 3B) of Adduct 4 confirms this assignment with an $(M-H)^-$ ion at *m/z* 380. Positive and negative ion FAB spectra

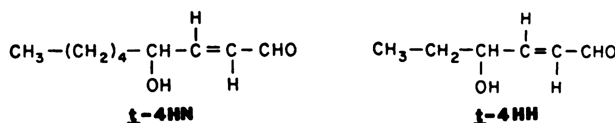

Fig. 1. Structures of 4-hydroxyalkenals studied.

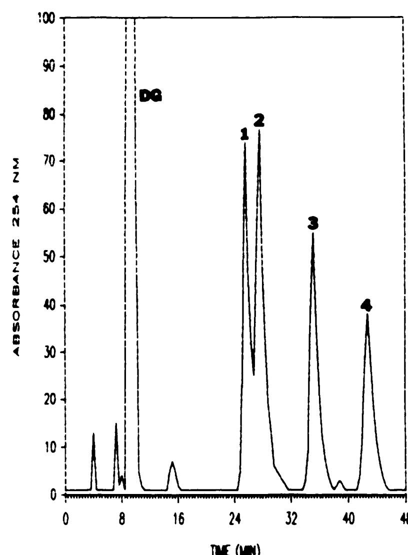

Fig. 2. HPLC chromatogram of t-4HH-deoxyguanosine adducts using system 1 (*DG*, deoxyguanosine).

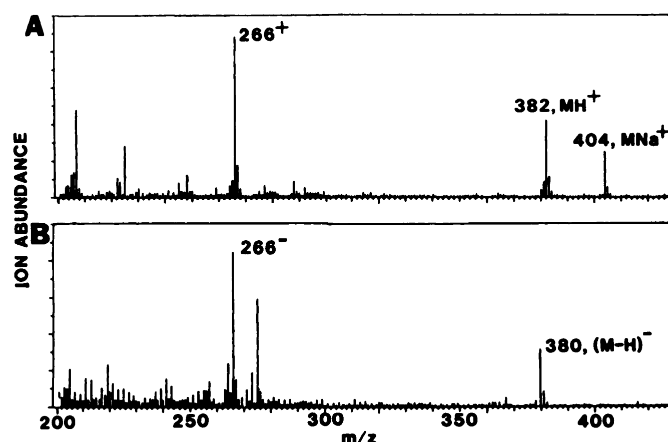

Fig. 3. Positive ion (*A*) and negative ion (*B*) FAB mass spectra for t-4HH-deoxyguanosine Adduct 4.

for the other t-4HH-deoxyguanosine adducts were nearly identical to those of Adduct 4.

The *m/z* 266 ion, common to the positive FAB spectra for all four t-4HH-deoxyguanosine adducts, was of special interest for further study by MS/MS. Accurate mass measurement indicated its origin exclusively by loss of deoxyribose from MH^+, accompanied by rearrangement of a single hydrogen (measured mass, 266.1264; $C_{11}H_{16}O_3N_5$ requires 266.1253, +4.2 ppm). In view of success by others in differentiating stereoisomers by MS/MS (31) we subjected the m/z 266 ion of all four adducts to collisionally activated decomposition using the MS/MS instrument. The resulting secondary mass spectra, although rich in structural detail, were identical, as were the collisionally activated decomposition spectra of MH^+ ions, and suggested the same skeletal arrangements in the four adducts.

The 360-MHz NMR spectrum of Adduct 1 is illustrated in Fig. 4 and proton NMR chemical shifts for all four adducts are given in Table 1.

The assigned chemical shifts for Adduct 1 are consistent with the structure shown in Fig. 4. The singlet observed for the C-8 hemiaminal proton at 6.28 ppm occurs only if the proton occupies an equatorial position which would prevent its coupling with the C-7 methylene protons. The hemiaminal proton

Downloaded from http://aacrjournals.org/cancerres/article-pdf/46/11/5682/2425429/cr0460115682.pdf by guest on 01 December 2023

Table 1 *360-MHz NMR spectral data for t-4HH-deoxyguanosine adducts*

Adducts 1 and 2   Adducts 3 and 4

Position	Chemical shifts (ppm)	
	Adducts 1 and 2	Adducts 3 and 4
2	7.89, s[a]	7.90, s
5	6.61, bs[b]	6.67, bs[b]
6	3.47–3.62, m	3.46–3.58, m
7	1.92, d; 1.60, t	2.00, d; 1.52, t
8	6.28, s	6.26, s
8-OH	7.45, s[b]	7.05, s[b]
11	3.47–3.62, m	3.46–3.58, m
11-OH	4.66, d[b]	3.38, d[b]
12	1.43, m	1.50, m; 1.33, m
13	0.92, t	0.93, t
1′	6.11, t	6.12, t
2′	2.54, m; 2.19, m	2.55, m; 2.20, m
3′	4.34, m	4.35, m
4′	3.80, m	3.81, m
5′	3.47–3.62, m	3.46–3.58, m
3′-OH	5.25, s[b]	5.26, s[b]
5′-OH	4.93, s[b]	5.03, s[b]

[a] s, singlet; d, doublet; t, triplet; m, multiplet; bs, broad singlet.
[b] Disappeared upon deuterium oxide treatment.

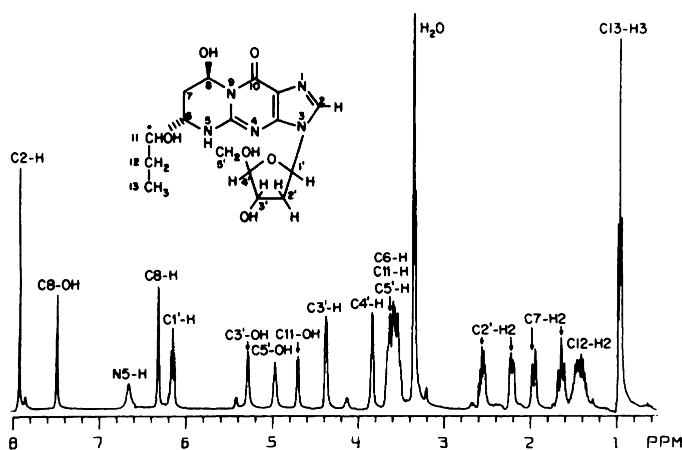

Fig. 4. The 360-MHz NMR spectrum for t-4HH-deoxyguanosine Adduct 1.

was assigned to the C-8 carbon rather than the C-6 carbon after comparing chemical shift values with those in the literature for similar molecules. Chung and Hecht (23) reported a chemical shift of 6.20 ppm for the C-8 hemiaminal proton of deoxyguanosine-crotonaldehyde adducts while Chung *et al.* (24) reported a shift of 5.0 ppm for the C-6 hemiaminal proton of deoxyguanosine-acrolein adducts.

The chemical shifts of the C-7 methylene protons, which were assigned to the doublet at 1.92 ppm and the triplet at 1.60 ppm, were also similar to the findings of Chung and Hecht (23) and led to the conclusion that the C-6 proton occupied an axial position as evidenced by its coupling with the 1.60-ppm methylene proton. The assignments of all C-H protons were confirmed using two-dimensional Fourier-transform NMR techniques.

The proton signals at 7.45, 6.61, 5.25, 4.93, and 4.66 ppm all disappeared following the addition of deuterium oxide, confirming the assignments of these protons as hydroxyl or amine protons. The signal at 7.45 ppm was concentration independent and shifted to 7.35 ppm at 40°C, suggesting that this proton was involved in an intramolecular hydrogen bond. This signal was assigned to the C-8 hydroxyl proton which would be expected to form hydrogen bonds with the C-10 carbonyl.

Adducts 1 and 2 exhibited identical NMR spectra, as did Adducts 3 and 4, and only slight differences were observed between the two pairs of adducts. The major difference in the NMR spectra between Adducts 1 and 2 and Adducts 3 and 4 was in the chemical shift of the C-8 hydroxyl proton, seen at 7.45 ppm for Adducts 1 and 2 and 7.05 ppm for Adducts 3 and 4. Molecular models demonstrated that the distance between the C-8 hydroxyl and the C-10 carbonyl was smaller when the axial C-8 hydroxyl was oriented above the purine plane than when it was below the plane. This would presumably lead to increased hydrogen bonding and a greater downfield shift (32) for the hydroxyl group located above the plane. Thus, the C-8 hydroxyl group with a chemical shift of 7.45 ppm was considered to reside above the purine plane and was assigned to Adducts 1 and 2. Differences in chemical shifts of this nature have been reported previously by Galliani and Pantarotto (22).

The differences between Adducts 1 and 2 are attributed to their existence as diastereomers at C-11, which would be expected since racemic t-4HH was used as a starting material in the reaction. This also explains the structural differences between Adducts 3 and 4. Further evidence to support these conclusions came from the formation of only two adducts in the reaction of achiral *trans*-2-hexenal with deoxyguanosine.

***Trans*-4-Hydroxy-2-nonenal-Deoxyguanosine Adducts.** Similar adducts were formed in the reaction of t-4HN with deoxyguanosine. HPLC analysis using System 2 revealed the presence of four adducts in both the small scale and preparative reaction mixtures and FAB mass spectrometry demonstrated that all four adducts had molecular weights of 423. Positive ion FAB spectra exhibited fragments at m/z 308 corresponding to the loss of deoxyribose and rearrangement of a proton which were analogous to the fragments at m/z 266 for the t-4HH-deoxyguanosine adducts. The NMR spectra for Adducts 1 and 2 of t-4HN differed from that reported for t-4HH Adducts 1 and 2 due to the additional methylene protons of t-4HN. Adducts 3 and 4 of t-4HN similarly corresponded to t-4HH Adducts 3 and 4.

DISCUSSION

The experimental results demonstrated that t-4HH and t-4HN alkylate deoxyguanosine *in vitro* under physiological conditions. These covalent interactions may provide additional information regarding the mechanisms for the genotoxicity of 4-hydroxyalkenals. Since t-4HH has been detected as a microsomal metabolite of the macrocyclic pyrrolizidine alkaloid senecionine (12), these results also suggest another possible mechanism for pyrrolizidine alkaloid genotoxicity.

The proposed reaction mechanism, shown in Fig. 5 for t-4HH, involves a Michael addition of the N^2 amino group of deoxyguanosine at the C-3 position of t-4HH. The Michael product rapidly cyclizes at the 1-N site of deoxyguanosine to form two pairs of diastereomeric adducts.

Similar types of deoxyguanosine adducts have been formed by reaction with acrolein and crotonaldehyde (22–24). In addition, Chung *et al.* (24) reported that acrolein also formed adducts in which a Michael addition occurs at position 1-N with subsequent cyclization at N^2. No adducts of this nature were detected with t-4HH and t-4HN and our results are in

Downloaded from http://aacrjournals.org/cancerres/article-pdf/46/11/5682/2425429/cr0460115682.pdf by guest on 01 December 2023

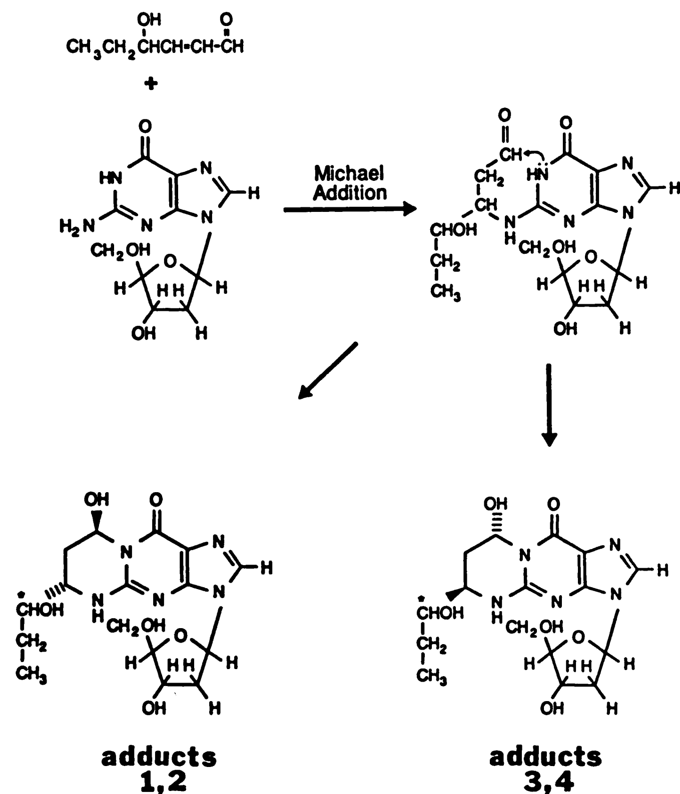

Fig. 5. Proposed reaction mechanism for formation of t-4HH-deoxyguanosine adducts.

agreement with investigators who did not detect similar adducts with crotonaldehyde (23). Steric crowding from the hydroxypropyl and hydroxyhexyl groups, respectively, would be expected to prevent formation of such adducts.

Prior results from our laboratory of an *in vivo* covalent binding study using t-4HH tritiated at the γ-position failed to detect significant binding of t-4HH to DNA or RNA in the livers of male rats over a 16-h period (33). Because the *in vitro* incubation of t-4HH showed a low yield of adduct formation (0.91%) after 16 h, it is not surprising that covalent binding would be difficult to detect. Additionally, since α,β-unsaturated aldehydes react more readily with low molecular weight molecules containing sulfhydryl groups such as glutathione than with amino groups (30, 34), it may be assumed that small amounts of t-4HH would be available for binding to DNA or RNA *in vivo*.

However, this low potential for binding may still be significant. Studies in our laboratory using isolated hepatocytes showed dose related genotoxic responses for t-4HH and t-4HN as measured by autoradiographic detection of unscheduled DNA synthesis (3).

The major route of detoxification for α,β-unsaturated aldehydes is through sulfhydryl conjugation (35). Esterbauer *et al.* (34) demonstrated that 4-hydroxyalkenals exhibited greater reactivity with glutathione and formed more stable conjugates than crotonaldehyde. It is postulated that the electron withdrawing 4-hydroxy group makes carbon 3 of the 4-hydroxyalkenal more electropositive and thus more susceptible to nucleophilic attack. The hydroxyl group also participates in the formation of stable hemiacetal conjugates which are less likely to dissociate to the starting or nonconjugated compounds (34).

Since glutathione may conjugate 4-hydroxyalkenals to a greater extent than crotonaldehyde, it is inferred that crotonaldehyde may exhibit higher carcinogenic potential. In the presence of glutathione depletion, 4-hydroxyalkenals may react more rapidly in Michael addition reactions and thus pose a greater carcinogenic threat than crotonaldehyde.

To determine the significance of the *in vitro* results of the binding of 4-hydroxyalkenals to deoxyguanosine, *in vivo* determination of adduct formation will be required. Future experiments should be performed in normal and glutathione depleted animals to determine the extent of protection from the genotoxic effects from t-4HH and t-4HN afforded by glutathione.

ACKNOWLEDGMENTS

We thank Dr. Christian Guenat of the National Institute of Environmental Health Sciences, Research Triangle Park, NC, for performing the MS/MS experiments.

REFERENCES

1. Marnett, L. J., Hurd, H. K., Hollstein, M. C., Levin, D. E., Esterbauer, H., and Ames, B. N. Naturally occurring carbonyl compounds are mutagens in *Salmonella* tester strain TA104. Mutat. Res., *148:* 25–34, 1985.
2. White, J. S., and Rees, K. R. The mechanism of action of 4-hydroxynonenal in cell injury. Chem.-Biol. Interact., *52:* 233–241, 1984.
3. Griffin, D. S., and Segall, H. J. Genotoxicity and cytotoxicity of selected pyrrolizidine alkaloids and alkenals. Toxicologist, *6:* 227, 1986.
4. Cadenas, E., Müller, A., Brigelius, R., Esterbauer, H., and Sies, H. Effects of 4-hydroxynonenal on isolated hepatocytes. Biochem. J., *214:* 479–487, 1983.
5. Wawra, E., Zollner, H., Schaur, R. J., Tillian, H. M., and Schauenstein, E. The inhibitory effect of 4-hydroxy-nonenal on DNA-polymerases α and β from rat liver and rapidly dividing Yoshida ascites hepatoma. Cell Biochem. Funct., *4:* 31–36, 1986.
6. Hauptlorenz, S., Esterbauer, H., Moll, W., Pumpel, R., Schauenstein, E., and Puschendorf, B. Effects of the lipid peroxidation product 4-hydroxynonenal and related aldehydes on proliferation and viability of cultured Ehrlich ascites tumor cells. Biochem. Pharmacol. *34:* 3803–3809, 1985.
7. Conroy, P. J. Carcinostatic activity of methylglyoxal and related substances in tumour-bearing mice. *In:* G. E. W. Wolstenholme, D. W. Fitzsimons, and J. Whelan (eds.), Submolecular Biology and Cancer, Ciba Foundation Series 67, pp. 271–300. Amsterdam: Excerpta Medica, 1979.
8. Conroy, P. J., Nodes, J. T., Slater, T. F., and White, G. W. Carcinostatic activity of 4-hydroxy-2-pent-en-1-al against transplantable murine tumour lines. Eur. J. Cancer, *11:* 231–240, 1975.
9. Benedetti, A., Comporti, M., and Esterbauer, H. Identification of 4-hydroxynonenal as a cytotoxic product originating from the peroxidation of liver microsomal lipids. Biochim. Biophys. Acta, *620:* 281–296, 1980.
10. Esterbauer, H., Cheeseman, K. H., Dianzani, M. U., Poli, G., and Slater, T. F. Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe^{2+} in rat liver microsomes. Biochem. J., *208:* 129–140, 1982.
11. Benedetti, A., Comporti, M., Fulceri, R., and Esterbauer, H. Cytotoxic aldehydes originating from the peroxidation of liver microsomal lipids. Identification of 4,5-dihydroxydecenal. Biochim. Biophys. Acta, *792:* 172–181, 1984.
12. Segall, H. J., Wilson, D. W., Dallas, J. L., and Haddon, W. F. *trans*-4-Hydroxy-2-hexenal: a reactive metabolite from the macrocyclic pyrrolizidine alkaloid senecionine. Science (Wash. DC), *229:* 472–475, 1985.
13. Bull, L. B., Culvenor, C. C. J., and Dick, A. T. The pyrrolizidine alkaloids. Front. Biol. *9:* 1–19, 1968.
14. McLean, E. K. The toxic actions of pyrrolizidine *(Senecio)* alkaloids. Pharmacol. Rev., *22:* 429–483, 1970.
15. Schoental, R. Toxicology and carcinogenic action of pyrrolizidine alkaloids. Cancer Res., *28:* 2237–2246, 1968.
16. Green, C. E., Segall, H. J., and Byard, J. L. Metabolism, cytotoxicity, and genotoxicity of the pyrrolizidine alkaloid senecionine in primary cultures of rat hepatocytes. Toxicol. Appl. Pharmacol., *60:* 176–185, 1981.
17. Williams, G., Mori, H., Hirono, I., and Nagao, M. Genotoxicity of pyrrolizidine alkaloids in the hepatocyte primary culture/DNA-repair test. Mutat. Res., *79:* 1–5, 1980.
18. Mattocks, A. R. and White, I. N. H. The conversion of pyrrolizidine alkaloids to *N*-oxides and to dihydropyrrolizine derivatives by rat-liver microsomes *in vitro*. Chem.-Biol. Interact. *3:* 383–396, 1971.
19. Mattocks, A. R. Toxicity of pyrrolizidine alkaloids. Nature (Lond.), *217:* 723–728, 1968.
20. Robertson, K. A. Alkylation of N^2 in deoxyguanosine by dehydroretronecine, a carcinogenic metabolite of the pyrrolizidine alkaloid monocrotaline. Cancer Res., *42:* 8–14, 1982.
21. Wickramanayake, P. P., Arbogast, B. L., Buhler, D. R., Deinzer, M. L., and Burlingame, A. L. Alkylation of nucleosides and nucleotides by dehydroretronecine; characterization of covalent adducts by liquid secondary ion mass spectrometry. J. Am. Chem. Soc., *107:* 2485–2488, 1985.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/46/11/5682/2425429/cr0460115682.pdf by guest on 01 December 2023

22. Galliani, G. and Pantarotto, C. The reaction of guanosine and 2′-deoxyguanosine with acrolein. Tetrahedron Lett., *24:* 4491–4492, 1983.
23. Chung, F. L. and Hecht, S. S. Formation of cyclic 1,N^2-adducts by reaction of deoxyguanosine with α-acetoxy-*N*-nitrosopyrrolidine, 4-(carbethoxynitroamino)butanal, or crotonaldehyde. Cancer Res., *43:* 1230–1235, 1983.
24. Chung, F. L., Young, R., and Hecht, S. S. Formation of cyclic 1,N^2-propanodeoxyguanosine adducts in DNA upon reaction with acrolein or crotonaldehyde. Cancer Res., *44:* 990–995, 1984.
25. Lutz, D., Eder, E., Neudecker, T., and Henschler, D. Structure-mutagenicity relationship in α,β-unsaturated carbonylic compounds and their corresponding allylic alcohols. Mutat. Res., *93:* 305–315, 1982.
26. Neudecker, T., Lutz, D., Eder, E., and Henschler, D. Crotonaldehyde is mutagenic in a modified *Salmonella typhimurium* mutagenicity testing system. Mutat. Res., *91:* 27–31, 1981.
27. Chung, F. L., Tanaka, T., and Hecht, S. S. Induction of liver tumors in F344 rats by crotonaldehyde. Cancer Res., *46:* 1285–1289, 1986.
28. Aberth, W., Straub, K. M., and Burlingame, A. L. Secondary ion mass spectrometry with cesium ion primary beam and liquid target matrix for analysis of bioorganic compounds. Anal. Chem., *54:* 2029–2034, 1982.
29. Erickson, B. W. γ-Hydroxy-α,β-unsaturated aldehydes via 1,3-bis-(methylthio)allyllithium: *trans*-4-hydroxy-2-hexenal. Org. Synth., *54:* 19–27, 1974.
30. Suyama, K., Tachibana, A., and Adachi, S. Kinetic studies on the reaction of α,β-unsaturated aldehyde with the amino group of glycine. Agr. Biol. Chem., *43:* 9–14, 1979.
31. Unger, S. E. Isomer differentiation in fast atom bombardment mass spectrometry/mass spectrometry. Int. J. Mass. Spectrom. Ion Proc., *66:* 195–208, 1985.
32. Silverstein, R. M., Bassler, G. C., and Morrill, T. C. Spectrometric Identification of Organic Compounds, Ed. 4, p. 194. New York: John Wiley & Sons, Inc., 1981.
33. Grasse, L. D., Lamé, M. W., and Segall, H. J. *In vivo* covalent binding of *trans*-4-hydroxy-2-hexenal to rat liver macromolecules. Toxicol. Lett., *29:* 43–49, 1985.
34. Esterbauer, H., Zollner, H., and Scholz, N. Reaction of glutathione with conjugated carbonyls. Z. Naturforsch. Teil Biochem. Biophys. Biol. Virol., *30:* 466–473, 1975.
35. Schauenstein, E., and Esterbauer, H. Formation and properties of reactive aldehydes. *In:* G. E. W. Wolstenholme, D. W. Fitzsimons, and J. Whelan (eds.), Submolecular Biology and Cancer, Ciba Foundation Series 67, pp. 225–244. Amsterdam: Excerpta Medica, 1979.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/46/11/5682/2425429/cr0460115682.pdf by guest on 01 December 2023