Persistence of Ethyl Carbamate-induced DNA Damage in Vivo as Indicated by Sister Chromatid Exchange Analysis

Mary K. Conner and Maria Cheng

Departments of Industrial Environmental Health Sciences [M. K. C.] and Biostatistics [M. K. C., M. C.], Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

ABSTRACT

Various treatment protocols were designed to investigate sister chromatid exchanges (SCEs) induced over successive posttreatment cell cycles in bone marrow and alveolar macrophage cells following treatment of C57BL/6J x DBA/2J F1 mice by i.p. injection of ethyl carbamate (3.3 mmol/kg). The same initial extent of alkylation in bone marrow and alveolar macrophages was suggested by identical SCE frequencies produced in both cell types by a one-cycle exposure protocol. The relatively lower responses in bone marrow cells by all other protocols may be a result of its faster mean population-cycling time.

Second- and third-division cell SCE data produced by the various protocols indicate persistence of SCE-inducing lesions with no evidence of repair. In spite of the demonstrated lack of repair, first-cycle ethyl carbamate treatment was less effective than was second-cycle treatment in inducing SCEs. These results could not be attributed to selection of less-damaged cells over 2 cycles or to enhanced bromodeoxyuridine sensitivity in the second-cycle treatment protocol. It is speculated that the apparent cancellation of SCEs occurring over two successive cycles in the two-cycle exposure protocol may indicate the transient presence of ethyl carbamate-induced DNA interstrand cross-links. A possible mechanism of action of ethyl carbamate involving the formation of a transient cross-link and a persistent DNA monoadduct is postulated.

INTRODUCTION

In spite of the widespread use of SCE3 as a sensitive assay for detection of potential mutagens and carcinogens (28, 31), a clear understanding of the relationship between SCEs and mutagenesis or carcinogenesis has not emerged. In studies where correlations have been demonstrated, quantitative relationships between induced SCEs and mutations were found to be dependent on specific chemical agents and genetic markers used (4, 21, 36).

Recent studies in our laboratory suggest that ethyl carbamate may be a particularly useful chemical model for investigating relationships between SCEs and tumorigenesis. Our findings of enhanced susceptibilities of alveolar macrophage and regenerating liver cells relative to bone marrow cells to ethyl carbamate-induced SCEs in vivo (7, 8) parallel previous descriptions of lung (19) and regenerating liver (10, 20) as highly susceptible target organs for ethyl carbamate-induced tumors. A similar mechanism for ethyl carbamate initiation of SCEs and lung adenomas is supported by the parallel appearance of the corresponding log-log dose-response curves and similar sensitivities demonstrated in the 2 assays (9). The remarkable similarities in relative potencies displayed by a series of carbamate esters as SCE inducers in alveolar macrophage cells (7, 9) and their reported activities in the lung adenoma assay are also consistent with a common etiology of carbamate-induced SCEs and tumorigenesis. Although all active carbamates produced comparable SCE frequencies in all cell types examined, enhanced (but not always statistically significant) responses were consistently observed in alveolar macrophages and regenerating liver relative to bone marrow cells (7, 9). While the enhanced SCE responses of alveolar macrophage and regenerating liver cells are suggestive of tissue susceptibilities similar to carbamate-induced tumorigenesis, the comparable magnitude of the bone marrow response indicates that the acute genotoxic effects of ethyl carbamate are not confined to the usual target tissue environments.

Because initial amounts of specific types of DNA lesions and the ability of the cell to repair DNA damage are factors critical to SCEs and cell transformation (32) as well as to carcinogenesis (30), the present study was designed to evaluate relative amounts and persistence of SCE-inducing lesions in alveolar macrophage and bone marrow cells.

As described in the present study, initial frequencies of induced SCEs in alveolar macrophages and bone marrow cells were determined following ethyl carbamate treatment at the beginning of the second cycle of BrdUrd incorporation. Two approaches were used to evaluate persistence of SCE-inducing lesions. Evaluation of reciprocal SCE frequencies in third-division cells, following ethyl carbamate treatment at the beginning of either the second or first cycle of BrdUrd incorporation, was used to detect the presence of persistent lesions over 2 to 3 cell cycles. In addition, unrepaird SCE-inducing lesions were examined in cells following approximate cell cycle interval delays between treatment and BrdUrd incorporation. Results of this investigation indicate that ethyl carbamate is unusually efficient in producing highly persistent SCE-inducing lesions. This property may be related to its tumorigenicity.

MATERIALS AND METHODS

Four-month-old C57BL/6J x DBA/2J F1 mice were used throughout the study. The parental breeders were purchased from The Jackson Laboratory, Bar Harbor, Maine.

All single acute injections of ethyl carbamate (99% pure; purchased from Aldrich Chemical Co.) dissolved in 0.9% NaCl solution were administered as an acute i.p. injection to groups of mice (3 to 8 mice/group) at a dose of 3.3 mmol/kg according to the protocols described in Chart 1. BrdUrd (10 mg/ml i.v.; flow rate, 3.6 ml/24 hr) was...
administered by continuous infusion using a simplified infusion device (i.e., C, 9-hr delay and C2, 20-hr delay). In all cases, colchicine injection and harvest of second (2nd M) and third (3rd M) division cells are at the indicated times. I, colchicine injection; H, harvest; M, division metaphase.

RESULTS

Selection of Cell Cycle Intervals. Control value relative percentages of first-, second-, and third-division cells, respectively, were: 17-hr BrdUrd infusion, bone marrow (27, 73, and 0%), alveolar macrophages (56, 44, and 0%), 28-hr BrdUrd infusion, bone marrow (6, 9, and 85%), and alveolar macrophages (34, 8, and 58%). The high percentage of first-division cells in the alveolar macrophage control distribution at the later harvest time suggests the presence of a second, more slowly cycling population or, perhaps, recruitment of new cells. Bone marrow is a nonsynchronous, heterogeneous cell population, and estimates of its mean cell cycle time vary considerably (16, 24, 35, 40). Although our data indicate that the bone marrow population cycles slightly faster than does the alveolar macrophage population, appropriate cell cycle intervals can be selected to assure that adequate yields of second- or third-division cells of both types are isolated simultaneously. In the present study, protocol timings were optimized for detection of cell populations having mean cell cycle times of approximately 8 to 12 hr. In the absence of significant alteration in cell kinetics of exposed populations, second- and third-division metaphases isolated at specified times, as described in the various protocols, are expected to be derived from similarly exposed populations.

Protocol Design. The protocols used in this study were designed to evaluate SCEs induced over successive cell cycles. SCE frequencies (induced plus base line) observed in second- and third-division alveolar macrophage and bone marrow cells following treatment of mice with ethyl carbamate (3.3 mmol/kg), according to the described protocols, are illustrated in Chart 2. All SCE frequencies in treated mice were significantly increased relative to the appropriate control. From these data, persistence of SCE-inducing lesions can be evaluated.

Visualization of SCEs via the BrdUrd labeling method depends upon the differential labeling of sister chromatids which is achieved by incorporation of BrdUrd into cellular DNA during at least the first of 2 replication cycles (22). Although SCEs may be induced during either cycle, none is visible until the second cycle. Consequently, because SCEs which occur during the first or second cycle cannot be distinguished from one another, second-division SCE frequencies are assumed to represent the total number of exchanges induced over both cell cycles (39). Thus, as illustrated in Table 1, examination of second-division cells from a population treated with ethyl carbamate midway between the 2 cycles (Protocol B) estimates SCEs induced during one posttreatment cycle, whereas treatment at the beginning of the first BrdUrd incorporation cycle (Protocol A) represents SCEs induced over Posttreatment Cycles 1 and 2. If, following treatment, BrdUrd infusion is delayed...
for 1 (Protocol C-1) or 2 (Protocol C-2) cell cycles, SCE frequencies in second-division cells represent those induced during Posttreatment Cycles 2 plus 3 or 3 plus 4, respectively.

Since detection of SCEs depends upon BrdUrd labeling of DNA, any exchanges induced during the delay cycle (prior to BrdUrd incorporation) will be lost, and only lesions remaining at the time of initiation of BrdUrd incorporation will contribute to SCEs induced over the subsequent cycles of BrdUrd incorporation. In addition, during the delay cycle, the number of SCE-inducing lesions remaining in a cell is simply reduced by one-half with each intervening cell division. Therefore, in the absence of DNA repair, SCE frequencies expected by the delay protocols can be calculated.

Because the number of lesions remaining at initiation of BrdUrd incorporation determines the number of induced SCEs (total minus base line), the frequency of induced SCEs is also expected to decrease by one-half with each intervening delay cycle, according to

$$2^{-c} \text{(SCE}_A - Z)$$

where c is the number of delay cycles, SCE_A is the total SCE frequency experimentally observed in the first-cycle treatment protocol, and Z is the experimentally observed base-line SCE frequency. The total SCE frequency (SCE)

expacted in the delay protocols is thus

$$\text{SCE}_c = 2^{-c} \text{(SCE}_A - Z) + Z$$

Any significant DNA repair will decrease the experimentally observed SCE frequencies in the delay protocols.

**Persistence of SCE-inducing Lesions as Determined in Second-Division Cells by Delay Protocols.** The experimentally observed second-division SCE frequencies in alveolar macrophages treated according to the delay protocols (C-1, 17.9 ± 1.9; C-2, 12.2 ± 1.0) are indistinguishable from those calculated in the preceding manner (C-1, 18.8; C-2, 11.7). These data suggest little or no repair of SCE-inducing lesions over at least 3 posttreatment cell cycles. Although a 1-cycle delay resulted in significantly lower (p < 0.01) than expected SCEs in bone marrow cells, a 2-cycle delay produced SCE levels equivalent to calculated levels (observed: C-1, 13.0 ± 1.0; C-2, 9.7 ± 1.2; expected: C-1, 16.4; C-2, 10.2). Nevertheless, the significantly elevated SCE frequency remaining in the 2-cycle delay protocol is consistent with unrepaird DNA lesions in bone marrow as well as alveolar macrophages.

**Persistence of SCE-inducing Lesions as Indicated by Reciprocal SCEs in Third-Division Cells.** Third-division cells are recognized by a staining pattern wherein approximately one-fourth of the total chromatins stains darkly, and three-fourths stain lightly. This unique staining pattern is a result of the random assortment and segregation of differentially labeled chromosomes from second-division cells into daughter cells such that, following a subsequent BrdUrd incorporation cycle, an average of one-half of the chromosomes are differentially labeled, and the remainder are fully substituted with BrdUrd. Consequently, SCEs in third-division cells appear as either reciprocal or nonreciprocal exchanges. Nonreciprocal SCEs are asymmetrical exchanges, visible in only one chromatid, and reflect SCE events which occur in the first and/or second cycles (39). By contrast, reciprocal SCEs, recognized as symmetrical exchanges between sister chromatids, are assumed to occur in the third cycle as a result of either new lesions or persistent lesions inducing exchanges for the first time during the third cycle.

Whether ethyl carbamate treatment was at the first (bone marrow, 5.8 ± 0.6; alveolar macrophages, 6.1 ± 0.7) or second (bone marrow, 5.2 ± 1.2; alveolar macrophages, 6.2 ± 0.4) cycle, identical reciprocal exchanges were observed in third-division cells. Persistence of SCE-inducing lesions, or lack of repair of induced lesions, over at least 3 cell cycles is evidenced by equally increased reciprocal exchanges in either bone marrow or alveolar macrophages, irrespective of treatment protocol. Since each successive posttreatment cell division reduces the number of DNA lesions by one-half the effective number of lesions potentially able to induce reciprocal SCEs in third-division cells treated at the first cycle is one-fourth of the original number of lesions initially present. In third-division cells treated at the second cycle, the total number of remaining lesions is one-half of the original number. However, due to loss of differential staining in the fully substituted regions, only those SCEs induced in the dThd-containing DNA regions will be visible, and because the dThd content of chromosomes exposed at the second cycle is only one-half of that present at the first cycle (see Table 1), the expected reciprocal SCE responses should be identical in the 2 protocols. An apparent lack of repair of induced lesions as indicated by the third-division reciprocal SCE data is in excellent agreement with the previously described delay protocol data.

**Relative SCE-inducing Efficiency of First-Cycle and Second-Cycle Treatment Protocols.** From Table 1, it follows that the frequency of SCEs observed in second-division cells, following treatment at the second cycle (SCE_p), represents exchanges induced during one posttreatment cycle (SCE_p) plus base-line SCEs (Z) which occur over 2 cycles of BrdUrd incorporation. This relationship can be expressed mathematically as

$$\text{SCE}_p = \text{SCE}_1 + \text{SCE}_2 + Z$$

Likewise, SCE frequencies produced in second-division cells following first-cycle treatment (SCE_p) can be expressed in terms of SCEs induced over 2 posttreatment cycles (SCE_1 + SCE_2) plus base-line SCEs as

$$\text{SCE}_p = \text{SCE}_1 + \text{SCE}_2 + Z$$

If no repair of SCE-inducing lesions occurs prior to the second cycle, the number of remaining lesions will be one-half of the number present at the first cycle.
M. K. Conner and M. Cheng

$SCE_2 = 0.5 SCE_1$

and

$SCE_4 = 1.5 SCE_1 + Z$

Substituting in our experimentally observed values for $SCE_e$ (bone marrow, 42.3 ± 3; alveolar macrophages, 42.3 ± 3.7) and $Z$ (bone marrow, 4.0 ± 0.4; alveolar macrophages, 4.8 ± 0.7), the expected values for $SCE_4$ are calculated to be: bone marrow, 61.5; alveolar macrophages, 61.1.

On the other hand, if efficient DNA repair occurs prior to the second cycle,

$SCE_2 = 0$

$SCE_4 = SCE_1 + Z$

and

$SCE_4 = SCE_e$

Thus, first cycle treatment should produce SCE frequencies equal to (complete repair) or greater than (persistent damage) the experimentally observed second-cycle treatment (24).

In the present study, first-cycle treatment was significantly less ($p < 0.005$) effective in inducing SCEs (bone marrow, 28.9 ± 4.2; alveolar macrophages, 32.8 ± 4.8) than was second-cycle treatment. Likewise, the cumulative SCE frequency distributions differed for the 2 protocols (Kolmogorov-Smirnov 2-sample test). However, similarly shaped distributions are apparent when the entire second-cycle treatment protocol distribution is shifted 6 to 10 SCEs to the left (a 10-SCE shift is illustrated for alveolar macrophages in Chart 3). SCE frequencies observed in the first-cycle treatment protocol are even lower than those expected for efficient DNA repair. In view of the fact that our data indicate little or no repair of SCE-inducing lesions, the differences between our experimentally observed and the respective expected second-cycle cell data are of too great a magnitude to be attributed to time of harvest relative to treatment (5). In fact, a highly significant cancellation of SCEs over 2 cell cycles is apparent.

**Evaluation of Cytotoxicity.** Significant cytotoxicity may be expressed as selection of “less-damaged” (as indicated by lower SCE levels) cells over 2 cycles in the first-cycle treatment protocol. However, we observed no evidence of cytotoxicity; such reduction in mitotic yield; or shift in distribution of first-, second-, or third-division cells (35) in treated mice relative to control mice.

Our third-division cell data also indicate a lack of ethyl carbamate-induced cytotoxicity. Nonreciprocal SCEs in third-division cells presumably occur during the first and/or second cell cycle. Thus, nonreciprocal SCEs should be identical to second-division SCE frequencies if they are progeny of the same initially exposed population. In fact, whether ethyl carbamate treatment was at the first or second cycle, excellent agreement was observed between cell mean SCE frequencies in second-division alveolar macrophage cells (first-cycle treatment, 32.8 ± 4.8; second-cycle treatment, 42.3 ± 3.7) and nonreciprocal SCEs in their respective third-division cells (first-cycle treatment, 32.3 ± 3.3; second-cycle treatment, 42.2 ± 2.0).

In bone marrow cells treated at the first cycle, lower (although not significantly so) nonreciprocal SCEs (23.0 ± 7.8) and cumulative SCE frequency distributions were observed in third-division cells relative to SCE levels in second-division cells (28.9 ± 4.2). Excellent agreement of SCE frequencies and cumulative frequency distributions were seen in second-division alveolar macrophage cells (first-cycle treatment, 42.3 ± 3.0) and third-division (41.8 ± 2.2; nonreciprocal SCEs) cells following treatment at the second cycle. In general, the same SCE trends observed in third-division (nonreciprocal SCEs) and their respective second-division cells are consistent with low cytotoxicity. No selection of less-damaged bone marrow or alveolar macrophage cells is indicated, since second-division cells containing mean SCEs as high as 30 to 40/cell can complete a subsequent cell division with no reduction in SCE frequency.

**BrdUrd versus dThd-containing DNA Sensitivity to Ethyl Carbamate Alkylations.** An obvious way in which second-cycle treatment differs from all other protocols is that the DNA duplex at the time of exposure contains one BrdUrd-substituted strand and one dThd-substituted strand (Table 1). Consequently, considerably enhanced sensitivity of BrdUrd-substituted DNA to ethyl carbamate alkylation may be the reason for our observation of greater effectiveness of second-cycle treatment relative to first-cycle treatment in inducing SCEs in second-division cells and nonreciprocal exchanges in third-division cells. Therefore, experiments were designed to evaluate the relative sensitivity of BrdUrd- and dThd-containing DNA to alkylation by ethyl carbamate.

As described previously, reciprocal SCE frequencies in third-division cells are independent of exposure at first or second cycle since, following 3 cycles of BrdUrd incorporation, only those SCEs induced in dThd-containing DNA regions are visible. Thus, reciprocal SCEs in third-division cells labeled with BrdUrd for all 3 cycles are indicative of the sensitivity of the original exposed dThd strand. Similarly, sensitivity of the original exposed (at the second cycle) BrdUrd strand can be

---

Chart 3. SCE frequency distributions in second-division alveolar macrophage cells from C57BL × DBA/2J F1, mice treated with ethyl carbamate (3.3 mmol/kg) at the beginning of the first (Protocol A) cycle or midway between the first and second (Protocol B) cycles of BrdUrd infusion.
evaluated using third-division cells labeled by an alternative method. Third-division cells which have incorporated BrdUrd for one cycle only, followed by a 2-cycle dThd chase, will demonstrate a "negative" staining pattern, i.e., three-fourths of the chromatin is darkly stained, and one-fourth is lightly stained, as compared to that of previously described third-division cells. In this manner, only SCEs induced in the BrdUrd-containing regions will be visible as reciprocal exchanges in third-division cells and, consequently, provide a measure of BrdUrd sensitivity. By using these 2 labeling methods for third-division cells and the second-cycle exposure protocol, an estimate of BrdUrd and dThd sensitivities can be made. However, regardless of the labeling method, because DNA is equally substituted with BrdUrd at the time of treatment, non-reciprocal SCEs should be identical in both third-division cell-labeling methods.

In Table 2, data are presented for third-division alveolar macrophage cells labeled by the described methods and treated at the second cycle. In order to achieve good-quality differential staining in the dThd chase method, the BrdUrd concentration in the infusion solution had to be increased (12 mg/ml) over normal (10 mg/ml). The increased BrdUrd concentration produced a decrease in both reciprocal and non-reciprocal SCEs in third-division cells labeled with BrdUrd for 3 cycles. It remains to be determined whether this general decrease is due to the increased BrdUrd in the infusion solution or that incorporated into DNA. Nevertheless, at the 12-mg/ml BrdUrd concentration, regardless of the labeling method, similar non-reciprocal SCE frequencies were produced in third-division cells. Enhanced BrdUrd-substituted DNA sensitivity is not indicated, since the dThd chase method produced slightly lower (although not significantly so) reciprocal SCEs than did 3-cycle BrdUrd labeling. If anything, there is a reduced induction of SCEs at higher concentrations of BrdUrd, which supports the idea that BrdUrd is not increasing the sensitivity of the chromosomes to ethyl carbamate.

**DISCUSSION**

The present study was designed to evaluate relative amounts of initial DNA alkylation and persistence of SCE-inducing lesions induced in alveolar macrophage and bone marrow cells by ethyl carbamate. The fact that second-cycle treatment produced identical SCE frequencies in the 2 cell types indicates similar amounts of initial DNA alkylation. The relatively lower SCE frequencies produced in second- and third-division cells by all other protocols may simply be a consequence of the more rapidly cycling or heterogeneous character of bone marrow relative to the alveolar macrophage cell population. Differences in cell kinetics may be involved in our consistent observation of slightly greater alveolar macrophage tissue sensitivity, since SCEs are dependent on number of lesions persisting until synthesis (41) and are, particularly in the absence of repair, expected to be dependent on the kinetics of formation of DNA adducts.

Various treatment protocols were used to demonstrate the highly persistent nature of ethyl carbamate-induced DNA damage as measured by SCE analysis in murine alveolar macrophage and bone marrow cells. Persistence of ethyl carbamate-induced lesions were made apparent as continued increases in SCE frequencies expressed in second-division cells over 3 to 4 successive cell cycles as well as increased reciprocal exchanges in third-division cells. In view of this evidence, which suggests little or no repair of SCE-inducing lesions, it was surprising that ethyl carbamate treatment at the first BrdUrd incorporation cycle was less effective in producing SCEs than was second-cycle treatment. If all induced and base-line SCEs in second-division cells are simply additive over the 2 cell cycles, first-cycle treatment should produce SCE frequencies equal to (if DNA repair is complete within one cycle) or greater than second-cycle treatment (24).

Such factors as selection against more highly damaged cells over successive cycles, enhanced sensitivity of BrdUrd relative to dThd-containing DNA, and random variations in SCE frequencies as a function of cell harvest time as causes of our anomalous results are not supported by our experimental data. An alternative explanation is that all induced exchange events are not simply cumulative but may, in fact, effectively cancel one another over 2 cycles. It was previously suggested that a cancellation of SCEs will occur if cross-links are induced at the first cycle, persist, and induce another exchange at the same locus in the second cycle, thus reducing the number of visible SCEs in second-division cells (38). We previously invoked this rationale in our recent study of SCE induction by the bifunctional antitumor agent, BCNU, wherein BCNU produced SCE trends (first-cycle treatment SCE frequencies less than those in second-cycle treatment) similar to the present ethyl carbamate data (3). The suggestion of cross-links implies a bifunctional metabolite of ethyl carbamate.

If metabolic activation of ethyl carbamate proceeds via dehydrogenation to vinyl carbamate, as suggested by Dahl et al. (14, 15), it might be expected to produce bifunctional metabolites and DNA adducts analogous to those of vinyl chloride, another effective inducer of SCEs in vivo (2). The dehydrogenation activation pathway for ethyl carbamate is, in fact, supported by our previous in vivo SCE studies wherein the unsaturated tumorigenic vinyl and allyl carbamates induced significant SCE responses at approximately 30- and 10-fold lower doses, respectively, than those of their corresponding saturated counterparts, i.e., ethyl and isopropyl carbamates (7).

Cross-linking of DNA has not specifically been proposed for vinyl chloride. However, its bifunctional metabolites, chloroethylene oxide and chloroacetaldehyde, as well as their nucleic acid alkylation products, have been characterized. The primary RNA alkylation products are 3,N'-ethenocytosine and 1,N'-ethenoadenine (25, 26), which are also formed to some extent

---

**Table 2**

*Comparison of ethyl carbamate-induced reciprocal and nonreciprocal SCEs in third-division alveolar macrophage cells labeled by 2 methods*

Mice were infused for 3 consecutive cycles (28 hr) with BrdUrd (10 or 12 mg/ml i.v.; flow rate, 3.6 ml/24 hr) or, alternatively, for 1 cycle (9 hr) with BrdUrd (12 mg/ml) followed by 2 cycles (19 hr) with dThd (7.9 mg/ml). In either case, ethyl carbamate (3.3 mmol/kg) was administered as an acute i.p. injection after the first cycle. Colchicine was administered after the third cycle and was followed 4 hr later by cell harvest.

<table>
<thead>
<tr>
<th>BrdUrd concentration (mg/ml) in infusion solution</th>
<th>Substitution of DNA duplex at exposure</th>
<th>Cycles of incorporation of SCEs in third-division alveolar macrophages</th>
<th>Nonreciprocal</th>
<th>Reciprocal</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 dThd, BrdUrd</td>
<td>1, 2, 3</td>
<td>42.2 ± 2.0*</td>
<td>6.2 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>12 dThd, BrdUrd</td>
<td>1, 2, 3</td>
<td>37.0 ± 2.6</td>
<td>4.1 ± 0.6</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± S.D.*
in vitro in "partially" denatured DNA (27). Likewise, after long-term (2 years) chronic exposure of rats to vinyl chloride in their M. K. Conner and M. Cheng
rat liver DNA produced by ethyl carbamate in vivo (34). Furthermore, Ribovich et al. (33) reported isolation of ethyl carbamate-induced RNA adducts identical to those of vinyl chloride. A possible mode of action of vinyl chloride-, vinyl carbamate-, and possibly ethyl carbamate-derived metabolites is suggested by an extension of a mechanism previously proposed (23, 29) for BCNU which produces nucleic acid adducts (N²-β-chloroethylguanine and 3,N⁴-ethenocytosine) which are saturated analogues of the adducts previously described for vinyl chloride. The multistep mechanism involves: (a) initial alkylation of guanine in one DNA strand; (b) reaction of the modified strand to form an interstrand cross-link with either its complementary base or an adjacent base on the complementary strand; and finally (c) intramolecular cyclization of the pyrimidine side chain.

If 7-N(2-oxoethyl)guanine is the primary initial DNA modification by ethyl carbamate (34), 2 possible modes of cross-link formation can be postulated. The first involves direct formation of a Schiff base between the aldehyde moiety and a free amino group on a base in the complementary strand. Alternatively, the aldehyde may interact intramolecularly with the O² position of guanine to form the hemiacetal, O⁶-7(1-hydroxyethane)-guanine (34). In turn, the hemiacetal may, in a manner analogous to the methyl derivatives of nucleic acids and formaldehyde (6), cross-link DNA via reaction with a free amino group on the complementary chain. In either case, due to the relatively short intermolecular distance involved, formation of cross-links may be favored under conditions of hydrogen-bond disruption, e.g., during DNA replication in dividing cells. A cross-link to cytosine is expected to be transient in that the guanine end of the cross-link can function as a leaving group in an intramolecular cyclization reaction leading to the final monoadduct. The aromatic character of the suspected persistent monoadduct, ethenodeoxycytosine, should ensure its facile formation and stability. The overall mechanism represents the conversion of a pyrimidine to a flat planar molecule of the same molecular size as that of a purine. The presence of such etheno derivatives in synthetic nucleic acids has been associated with misincorporation of bases during DNA synthesis (1, 18) and RNA transcription (37) in vivo. The carcinogenic potency of ethyl carbamate and vinyl chloride may be related to persistence of such potentially miscoding lesions. However, to our knowledge, no reports exist concerning repair or persistence of etheno derivatives in vivo.

The formation of transient monoadducts and cross-links is consistent with the present ethyl carbamate-induced SCE data. Cancellation of SCEs as observed in our study does not necessarily imply persistence of cross-links over 2 cycles. The transient cross-link monoadduct mechanism proposed here would also allow cancellation of SCEs. For example, if cross-links are present only at the first cycle and are converted to monoadducts prior to the second cycle, cancellation will still result due to a finite probability of induction of exchanges by persistent monoadducts at the same locus previously occupied by the cross-link. Clearly, further studies are required to determine whether the speculated transient intermediates and persistent DNA lesion are produced.

Although many questions remain unanswered regarding its mode of action, ethyl carbamate is an extremely interesting chemical to use in future studies concerning the mechanism of SCE. In turn, the experimental design presented here illustrates the potential utility of the SCE assay for investigating persistence of alkylated sites on DNA produced by other chemicals as well.

REFERENCES

12. Dahl, G. A., Miller, E. C., and Miller, J. A. Comparative carcinogenicities and mutagenicities of vinyl carbamate, ethyl carbamate, and ethyl N-hydroxy-

CANCER RESEARCH VOL. 43
Ethyl Carbamate-induced Persistent SCEs


Persistence of Ethyl Carbamate-induced DNA Damage \textit{in Vivo} as Indicated by Sister Chromatid Exchange Analysis

Mary K. Conner and Maria Cheng


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/43/3/965

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.