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O^6-Alkylguanine DNA Alkyltransferase Activity in Student Embalmers

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O^6-Alkylguanine-DNA alkyltransferase (AGT) activity was assessed in peripheral blood lymphocytes among 23 mortuary science students before and after 9 weeks in a laboratory course in techniques of embalming. Formaldehyde exposure was established by environmental monitoring. The average air concentration of formaldehyde during embalming was about 1.5 ppm. At the pre-exposure sampling, baseline DNA repair capacity tended to be reduced in subjects who reported a prior history of embalming ($p = 0.08$). From pre- to post-exposure, 17 subjects decreased in DNA repair capacity, while only 6 increased ($p < 0.05$). Analysis of variance, including adjustment for age, sex, and smoking status, confirmed these findings. Among the eight subjects who had no embalming experience during the 90 days before study, seven had decreased and one had increased AGT activity during the period of study ($p < 0.05$). For those with prior embalming experience, 10 subjects decreased in AGT activity, while 5 increased ($p < 0.05$). Although the major chemical exposure in embalming practice was to formaldehyde, no clear link was established between amount of formaldehyde exposure and AGT activity. Am. J. Ind. Med. 31:361–365, 1997.

KEY WORDS: formaldehyde; occupational exposure; DNA repair; embalming

INTRODUCTION

Occupational formaldehyde exposure has been linked in some studies to excesses of cancers of the nasopharynx, nose, and central nervous system and to excesses of leukemia, but the overall evidence in humans for the carcinogenicity of formaldehyde is limited [IARC, 1995, Blair and Kazerouni, 1996]. Formaldehyde is mutagenic [Auerbach et al., 1977] and causes nasal tumors and possibly tumors at other sites in rodents [IARC, 1995]. Although DNA–protein cross-links are suspected in formaldehyde carcinogenesis [Grafstrom et al., 1983; Casanova et al., 1989], other mechanisms may also be involved as suggested by reports that administration of formaldehyde increases the experimental tumor rate due to N-nitrosodimethylamine (NDMA), N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), and N-nitrosodiethylamine (NDEA) [IARC, 1995].

O^6-Alkylguanine-DNA alkyltransferase (AGT) is critical for the repair of DNA damage due to alkylating agents [Pegg et al., 1995]. In vitro studies indicate that formaldehyde inhibits AGT repair of N-methyl-N-nitrosourea (NMU)-induced O^6-methylguanine and potentiates the mutagenicity of NMU in human fibroblasts [Grafstrom, 1985]. Although the potential effect of formaldehyde on DNA repair capacity has been recognized for some time, no studies have evaluated this directly in humans. We have assessed O^6-AGT activity in peripheral blood lymphocytes among mortuary science students. An earlier investigation had shown increased buccal and lymphocyte micronuclei in this population [Suruda et al.,1993].

MATERIALS AND METHODS

Students in associate or bachelor's degree programs at a college of mortuary science in Cincinnati who were entering

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the initial laboratory course in embalming technique were enrolled for study. Blood samples were collected before the embalming course. Each subject also completed a questionnaire, concerning embalming experience and tobacco use. After 9 weeks of embalming practice, a second blood sample was drawn. During the 9-week period, industrial hygiene monitoring was performed and information was collected concerning formaldehyde exposure-related activities.

A Ficoll-hypaque isolation of peripheral blood lymphocytes was carried out at both collection times. For this, 40 ml of blood was mixed with an equal amount of Hank's solution at room temperature. The mixture was layered over 10 ml of lymphoprep medium, centrifuged, and the mononuclear cells collected from the interphase band. The cells were then washed in RMI-1640 medium containing antibiotics, assessed for viability and total number, and frozen as a wet pellet, at −70°C. The thawed samples were mixed with phenylmethylsulfonylfluoride to an end concentration of 1 mM and with pepstatine A to an end concentration of 2.5 µg/ml. The homogenization was carried out by sonication at 4°C. The cell homogenate was centrifuged at 10,000*g* for 6 min and the supernatant was frozen in 10-µl portions for the AGT assay.

The AGT analyses were done according to Klein and Oesch [1990]. As O^6- methylguanine (O^6-meG) is repaired by AGT in a 1 : 1 stoichiometric irreversible reaction, the concentration of the repair protein in cells can be quantitated by determination of the removal of the methyl group from O^6-meG in vitro. In this assay, lambda-phage DNA containing one ^{32}P-labeled O^6-meG in each *Bam*HI site was used as a substrate for the enzyme. After incubation of the sample and the labeled substrate, an additional proteinase K digest with 30 µg proteinase was performed for 1 hr at 55°C, facilitating the phenol extraction. All determinations were done in triplicate as sets of pre- and post-exposure pairs, with blinding for pre- or post-exposure status. Activity of O^6-AGT was measured as pmol AGT/mg protein (undetectable at <0.006 pmol AGT/mg protein). The protein determination was done by the BioRad protein assay [Bradford, 1976].

Details of the formaldehyde exposure assessment are provided elsewhere [Suruda et al., 1993]. For each embalming carried out at the school, personal formaldehyde exposure was measured using a PF-20 STEL passive monitor (Air Quality Research, Research Triangle Park, NC), at the breathing zone, and analyzed by the chromotrophic method [Katz, 1977]. In addition to the personal monitoring, short-term (peak) exposure measurements were performed using a continuous reading instrument (Interscan model 4160 SP, Chatsworth, CA). Students who had participated in embalmings during the 90 days before study were identified by questionnaire. Several students lived at funeral homes or had part-time jobs in funeral homes. These students were provided with the passive monitors and asked to follow the same procedures for measuring exposures during embalmings outside the college laboratory, although compliance could not be monitored. Also, students kept logs regarding the duration and type of each embalming, and the amount of solution used. Information from the passive monitors and from the embalming log was used to estimate cumulative formaldehyde exposure, in ppm-hr Σ [exposure in ppm $\times$ duration of exposure]), to formaldehyde during the study period.

AGT activity was measured at baseline and after 9 weeks in the embalming course. Correlations are given as the Spearman rank correlation. The association of selected factors with baseline AGT was determined by the Wilcoxon rank-sum test. For the statistical analysis of change in AGT between baseline and the second blood collection, each subject was used as his or her own control. Differences in pre- and post-exposure values for AGT were assessed by the Wilcoxon signed rank test and by analysis of variance for repeated measures [Hollander and Wolfe, 1973].

RESULTS

The subjects (n = 23) were predominantly male, aged <25 years, and nonsmokers. Fifteen of the students reported participating in embalming practice, ranging from two to greater than 90 embalmings, in the 90 days before the beginning of the course. Eight subjects reported no prior embalming experience.

DNA repair capacity at baseline tended to be reduced in subjects with a prior history of embalming ($p = 0.08$). When the number of embalmings during the previous 90 days was considered, however, no clear exposure response relationship with AGT activity was ascertained ($r = -0.29$, $p = 0.19$). Sex, age, and current tobacco use were not clearly related to pre-exposure DNA repair activity (Table I).

Post-exposure AGT activity was correlated with pre-exposure activity ($r = 0.42$, $p < 0.05$). From pre- to post-exposure, however, 17 subjects decreased in DNA repair capacity, while only 6 increased ($p < 0.05$). Analysis of variance, including adjustment for age, sex, and smoking status, confirmed these findings. As shown in Figure 1, among the eight subjects who had no embalming experience during the 90 days before study, seven (88%) had decreased and one had increased AGT activity during the period of study ($p < 0.05$). For those with prior embalming experience, 10 subjects (67%) decreased in AGT activity, while 5 increased ($p > 0.05$) (Fig. 1).

During the study period, most subjects performed five to nine embalmings, with a minimum of two and a maximum of 29. Eight of the study subjects reported having carried out additional embalmings outside the school during the 9-week study period, with the number of such embalmings ranging from 1 to 11. The average air concentration of formaldehyde during embalming was about 1.5 ppm. Short-

TABLE I. Baseline Characteristics of Students of Embalming Studied for DNA-Repair Activity

		AGT activity (baseline)[a]		
Trait	**n**	**Mean (SD)**	**Median**	**p-value[b]**
Sex				
Female	7	0.15 (0.08)	0.12	
Male	16	0.10 (0.07)	0.09	0.22
Age				
<25	14	0.11 (0.06)	0.10	
25+	9	0.14 (0.10)	0.12	0.57
Tobacco use (current)				
No	17	0.10 (0.07)	0.10	
Yes	6	0.16 (0.09)	0.17	0.26
Previous embalming				
No	8	0.16 (0.09)	0.18	
Yes	15	0.10 (0.06)	0.09	0.08

[a]pmol AGT/mg protein.
[b]Wilcoxon rank-sum test, with continuity correction of 0.5.

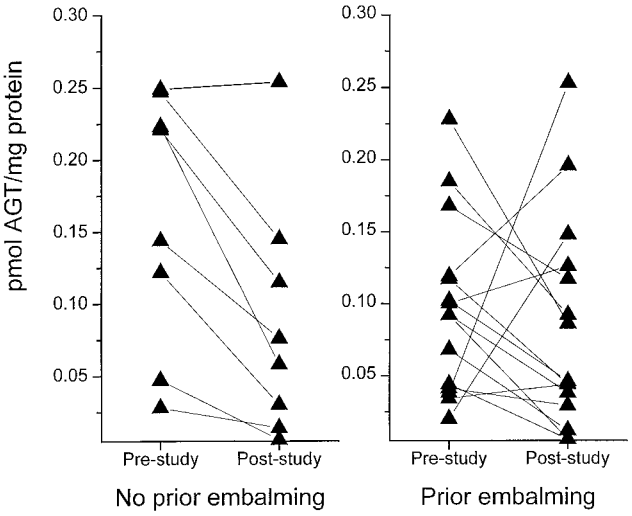

FIGURE 1. O^6-Alkylguanine-DNA alkyltransferase (AGT) activity in peripheral blood lymphocytes of students of embalming, at the beginning (pre-study), and after 9 weeks of training (post-study).

term exposure monitoring during some embalmings, however, showed peak exposures three to nine times that of the corresponding time-weighted average, while measurements for glutaraldehyde and phenol were below the limit of detection.

Total embalming-associated formaldehyde exposure during the study period, including embalmings done outside the school, were within the range of 5.7–82.0 ppm-hr (mean = 18.4, SD = 15.6). As expected, the total number of embalmings was correlated with the estimated total formaldehyde exposure ($r = 0.59$, $p < 0.01$). During the study period, subjects with prior embalming experience participated in a larger number of embalmings ($p = 0.09$) and had greater estimated exposure to formaldehyde during this time ($p < 0.05$).

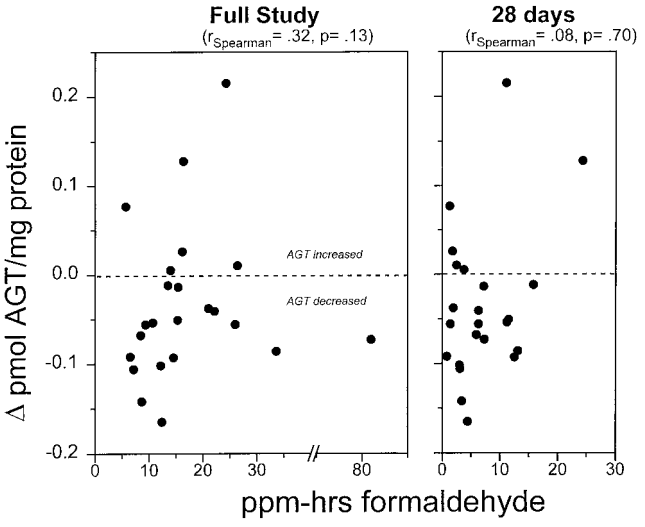

FIGURE 2. Cumulative formaldehyde exposure (ppm-hr), during 9 weeks and for the last 28 days, and change in AGT activity (Δpmol AGT/mg protein), between pre-study and post-study.

Although AGT activity tended to decrease after embalming practice, the extent of decrease could not be related to the extent of formaldehyde exposure throughout the 9-week period or during the last 28 days of the study period (Fig. 2).

DISCUSSION

In vitro studies indicate that formaldehyde inhibits the repair of N-methyl-N-nitrosourea (NMU) induced O^6-methylguanine and potentiates the mutagenicity of NMU in human fibroblasts [Grafstrom, 1985]. This is the first study to assess AGT activity in humans in relation to environmental formaldehyde exposure.

Embalming experience was associated with decreased DNA repair capacity; subjects who had previously embalmed had a tendency toward lower levels of AGT activity at baseline and AGT activity levels were decreased after 9 weeks of embalming practice, particularly among subjects with no prior embalming experience. These effects, however, could not be linked directly to the amount of formaldehyde exposure.

The study has several limitations. The study group was small and many of the subjects had previously embalmed. Although subjects with prior formaldehyde exposure had lower AGT activity at study entry, we could not evaluate the extent of exposure to formaldehyde during this period. Also, subjects who had previously embalmed tended to have greater formaldehyde exposure during the period of study,

tending to confound the statistical analysis. Further, we were unable to study the detailed temporal association between formaldehyde exposure and AGT activity. Studies are needed for better characterization of the short-term impact of embalming practice and associated formaldehyde exposure on AGT activity.

In our study, pre-exposure and post-exposure formaldehyde levels were correlated supporting earlier studies showing relative intra-individual stability of AGT activity in human lymphocytes [Sagher et al., 1989; Vahakangas et al. 1991] and bronchoalveolar lavage cells [Vahakangas et al., 1991]. We found no clear association of sex or age with AGT activity, although men and older subjects had somewhat reduced levels. Formaldehyde and other aldehydes are present in cigarette smoke [IARC, 1986], but the reported effects of tobacco use on AGT activity are inconsistent [Vahakangas et al., 1991; Klein and Oesch, 1990; Oesch and Klein, 1992; Drin et al., 1994]. This study showed a tendency toward increased AGT activity among smokers.

O^6-AGT repairs O^6-methylguanine. The repair process involves the direct stoichiometric transfer of the methyl adduct to a unique cysteine on AGT, resulting in a stable but inactive protein. Once depleted, AGT activity can occur only through AGT synthesis [Mitra and Kaina, 1993]. If not repaired before DNA replication, O^6-methylguanine adducts can result in G-to-A transitions [Ellison et al., 1989] and in mutations related to functional alterations in H-ras [Georgiadis et al., 1991] and p53 [Ohgaki et al., 1992]. Mutations at GC base pairs have been identified in p53 in formaldehyde-induced nasal squamous cell carcinomas in rats [Reico et al., 1992]. Also, transgenic mice expressing human O^6-alkylguanine-DNA alkyltransferase are protected from N-methyl-N-nitrosourea-induced thymic lymphomas [Dumenco et al., 1993].

Formaldehyde is highly reactive, binding readily to protein and DNA. Decreased AGT activity could result from protein inactivating cross-links or from inactivation of the enzyme by methylation of the active cysteine site [Grafstrom et al., 1983; Hemminki, 1982]. Decreased AGT activity has been shown in peripheral blood lymphocytes from patients treated with methylating chemotherapeutic agents [Sagher et al., 1988; Ayi et al., 1994], from clinical workers handling cancer chemotherapeutic agents, and from automobile workers exposed to rubber and tires [Oesch and Klein, 1992]. Because detoxification of formaldehyde is rapid, however, it remains to be shown experimentally that respiratory exposures could have a substantial impact on peripheral lymphocytes.

Excesses of nasal and nasopharyngeal cancer have been linked to formaldehyde exposure in some epidemiologic studies and some professionals exposed to formaldehyde as embalmers, pathologists, or anatomists have shown excesses of brain cancer and leukemia (IARC, 1995; Blair and Kazerouni, in press]. We did not study DNA repair capacity in the upper respiratory passages or at other possible sites for formaldehyde-associated carcinogenesis, but O^6-methyltransferase activity in lymphocytes and gastric mucosa is correlated [Kyrtopoulos et al., 1990], suggesting that measurements in lymphocytes may also be indicative of activity elsewhere.

In summary, AGT activity was reduced among students with embalming experience. Although the major chemical exposure in embalming practice is to formaldehyde, this study did not show a direct link between formaldehyde exposure and AGT activity.

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