Relevance of Environmental Alkylating Agents to Repair Protein O⁶-Alkylguanine-DNA Alkyltransferase: Determination of Individual and Collective Repair Capacities of O⁶-Methylguanine¹

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ABSTRACT

The repair capacity for O⁶-methylguanine was determined in cell homogenates of peripheral blood lymphocytes of 35 automobile industry workers, exposed to rubber and tires, and of 35 clinical workers, handling cancer chemotherapy agents, compared to control groups. λ-Phage DNA containing one ³²P-labeled O⁶-methylguanine in each BamHI site was used as substrate for the repair protein O⁶-alkylguanine-DNA alkyltransferase (AGT). The clinical personnel showed in the mean a highly significantly (P = 0.0014, Wilcoxon U test, Mann and Whitney) reduced activity of the repair enzyme [3.28 ± 0.28 (SEM) fmol AGT/ng DNA] as compared to 37 control persons (4.88 ± 0.32 fmol AGT/ng DNA). The mean AGT value of the automobile industry workers (4.40 ± 0.28 fmol AGT/ng DNA) was not significantly different (P = 0.1303) from the mean of 38 examined controls (5.00 ± 0.28 fmol AGT/ng DNA). By dividing these employees into subgroups according to their different work environments (handling of rubber fittings, tire mounting, and tire storage, respectively) the mean AGT value of the 15 tire storage workers was significantly lower (3.80 ± 0.36 fmol AGT/Mg DNA) than the mean value of the controls. The interindividual variations in the activity of AGT were 5.1- and 6.5-fold in the control groups and 5.6-fold respectively) the mean ACT value of the 15 tire storage workers was significantly lower (3.80 ± 0.36 fmol AGT/Mg DNA) than the mean value of the controls. The interindividual variations in the activity of AGT were 5.1- and 6.5-fold in the control groups and 5.6-fold for the automobile industry workers; the largest variations were found in the group of the clinical personnel with 12.6-fold. No significant correlations between AGT and age, sex, or smoking behavior were observed in any of the groups examined. The decrease in AGT activity will render the afflicted individuals more susceptible to further exposure to alkylating agents.

INTRODUCTION

Alkylating agents such as N-nitrosoamines react with DNA resulting in the formation of alkylated adducts at the N and O atoms of the purine and pyrimidine bases (1, 2). Every organism, including humans, dispose of a complex repair system, capable of removing and replacing or selectively eliminating these and other DNA lesions. Deficiencies in DNA repair enzymes may increase the cancer risk if the individual is exposed to chemicals which cause mutagenic DNA lesions. There is substantial evidence that O⁶-methylguanine is a major source of mutation if not repaired by AGT² in a 1:1 stoichiometric irreversible reaction (3–5). A number of studies on Chinese cancer patients compared to control populations suggest a direct association between environmental nitrosamine exposure, formation of O⁶-methylguanine, and development of cancer (6, 7).

In rubber and tire manufacturing as well as metalworking, relatively high concentrations of volatile N-nitroso compounds have frequently been found in the ambient air and in grinding fluids (8). Another exposed professional group is clinical personnel who mix and handle chemotherapeutics. The determination of AGT activity in peripheral blood lymphocytes of patients with leukemia showed lower activities of this repair enzyme in those patients who were treated with methylating chemotherapeutic agents such as procarbazine (9). Some of the clinical personnel we examined reported noticeable hair loss, which may imply considerable exposure of personnel to the drugs.

Recently we reported a highly sensitive assay for AGT to determine DNA repair capacities in small samples of blood (10). λ-Phage DNA containing specifically ³²P-labeled O⁶-mGua is used as substrate for the examination of the repair protein. In this study the developed method is applied to measure the individual and collective repair capacities of O⁶-mGua in human lymphocytes of employees of an automobile company working with rubber or tires and of clinical personnel handling chemotherapeutic agents compared to control persons.

MATERIALS AND METHODS

All chemicals, including the modified nucleotides 5'-d-O⁶-meGTP and 3'-d-O⁶-meGMP were obtained as described previously (10). Assay for O⁶-Alkylguanine-DNA Alkyltransferase. The preparation of λ-Phage DNA containing O⁶-mGua in each BamHI site, the assay of AGT in cell homogenate corresponding to 500,000 cells with this modified DNA, as well as the high pressure liquid chromatography analysis were done as described previously (10). All determinations were carried out in triplicate.

Cell Homogenates of Human Lymphocytes. Blood samples from 10 ml of the exposed and control individuals were transported in heparinized tubes on ice for lymphocyte isolation according to Ref. 11 by Ficoll-metrizoate centrifugation. The collected cells were resuspended in 50 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol, 0.1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride to a concentration of 10⁷/ml. The homogenization was carried out by sonication at 4°C (20 × 0.5-s pulses).

Probands. Thirty-five male and female persons of an automobile company working in different departments (tire storage, tire mounting, and workers handling small rubber parts) were examined and compared to 38 male and female persons without any known exposure to alkylating agents. The AGT levels of 35 female and male clinical workers of three different hospitals, handling chemotherapeutics, were compared with the levels of 37 control persons. Information on the age, occupation, current smoking and alcohol drinking habits was obtained for all subjects.

Statistical Analysis. The statistical analysis was performed by using the two-sided Wilcoxon U test, Mann and Whitney. A difference was considered as significant if the corresponding P value was less than 0.05.

RESULTS

The examined employees of the automobile company showed a mean of 24,139 ± 1,505 cpm (SEM) increase of radioactive guanosine monophosphate after incubation with AGT from lymphocyte homogenate containing 500,000 cells. This corre-
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The decrease of [³²P-dO⁶-meGMP, exactly equivalent to the increase of [³²P-dGMP, in the 35 subjects. The interindividual variations in the activity of AGT were 5.6-fold. The mean value of the whole group did not differ significantly (P = 0.1303) from the mean of the examined control group, which was 27,316 ± 1,635 cpm, corresponding to 5.00 ± 0.28 fmol AGT/µg DNA. The individual determinations of the 38 nonexposed male and female people are shown in Fig. 2. In this group the interindividual variations of AGT activity were 6.5-fold. After dividing the subjects into subgroups according to their different work environments, the resulting means of the AGT capacity were: 6 females working with small rubber parts (armament): 23,067 ± 5,025 cpm (± 4.20 ± 0.92 fmol AGT/µg DNA); 15 males at tire mounting: 28,086 ± 1,896 cpm (± 5.12 ± 0.36 fmol AGT/µg DNA); 15 males in tire storage (one man was working both in mounting as well as in storage and was considered in both groups): 20,819 ± 1,928 cpm (± 3.80 ± 0.36 fmol AGT/µg DNA). The mean values of the workers in armament and tire mounting showed no significant difference (P = 0.3926 and P = 0.9842) to the mean of the control group. However, the workers in tire storage showed a significantly lower (P = 0.0270) repair capacity of O₆-methylguanine than the control group.

The mean AGT activity of the examined clinical workers, handling chemotherapeutic agents, was significantly lower (P = 0.0014) than that of the compared controls; both groups were composed of males and females. Fig. 3 shows the individual determinations of the exposed group, ranging from 3,342 to 42,092 cpm, with a mean of 18,081 ± 1,580 cpm, corresponding to 3.28 ± 0.28 fmol AGT/µg DNA and interindividual variations of 12.6-fold. The values of the control collective are given in Fig. 4. The mean of this group was 26,787 ± 1,762 cpm, corresponding to 4.88 ± 0.32 fmol AGT/µg DNA. The interindividual variations in the activity of the repair protein were 5.1-fold in this group.

The variations of the examined AGT activity between the triplicate determinations were less than 13%. Eight blood samples of two control persons over a period of 2 years showed intra-individual variations of 1.0- to 1.6-fold in AGT content, which are much lower than the interindividual variations in any one of the examined groups.

No significant correlations between AGT and age, sex, or smoking behavior were observed in any group examined.

DISCUSSION

In the present study we measured the individual repair capacities of AGT of two professional groups, exposed to different alkylating, O₆-meG generating agents in comparison to control groups. The recently developed assay (10) proved highly sensitive, 10⁻¹⁶ mol of the adduct were easily detectable. One step in our synthesis strategy includes the blunt end ligation of DNA fragments with an O₆-mGua-T base pair near their ends. Because blunt end ligations never yield 100%, variations in the resulting DNA fragment lengths are unavoidable. Therefore it is necessary to perform the AGT determinations of the probe bands in parallel to the comparable group with the same substrate DNA; only these two groups are comparable and statistically evaluable with each other. However both examined control groups showed nearly the same mean AGT activity with interindividual variations in the same range, indicating that the synthesized DNA is nearly identical in length and superstructure and represents a good substrate for this AGT assay.

The AGT activity in peripheral blood lymphocytes found by other investigators also showed great interindividual variations.
In conclusion, our findings suggest a reduced AGT activity in peripheral blood lymphocytes in professional groups exposed to O^-guanine alkylating agents. These results are underlined by the discovery of O^-mGua in DNA obtained from individuals exposed to environmentally derived alkylating agents (15, 16).

Further investigation of other occupationally and environmentally exposed groups is needed to confirm the reducing effect of alkylating agents in the surroundings on the repair enzyme AGT. A reduced repair capacity would increase the cancer risk of an individual if further exposed to alkylating agents.

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