O\textsuperscript{6}-Alkylguanine-DNA Alkyltransferase Activity in Normal Human Tissues and Cells

Roland C. Graffstrom,\textsuperscript{1} Anthony E. Pegg,\textsuperscript{2} Benjamin F. Trump, and Curtis C. Harris\textsuperscript{3}

Laboratory of Human Carcinogenesis, National Cancer Institute, NIH, Bethesda, Maryland 20205 [C. C. H.]; Department of Physiology and Specialized Cancer Research Center, The Milton S. Hershey Medical Center, Hershey, Pennsylvania 17033 [A. E. P.]; Department of Forensic Medicine, Karolinska Institutet, S-104 01 Stockholm 60, Sweden [R. C. G.]; and Department of Pathology, University of Maryland, School of Medicine, Baltimore, Maryland 21201 [B. F. T.]

ABSTRACT

Normal adult human tissues and cultured bronchial epithelial cells and fibroblasts exhibit O\textsuperscript{6}-alkylguanine-DNA alkyltransferase activity in vitro by catalyzing the repair of the promutagenic alkylation lesion O\textsuperscript{6}-methylguanine from DNA. The amount repaired by extracts of liver, peripheral lung, and colon extracts was proportional to the amount of extract protein. Repair of O\textsuperscript{6}-methylguanine led to stoichiometric regeneration of guanine in the DNA and stoichiometric formation of S-methylcysteine in protein. Alkyltransferase activity varies in the different human tissues tested in the decreasing order of liver > colon > esophagus > peripheral lung > brain. Extracts of lung tissues, cultured tissues tested in the decreasing order of liver > colon > esophagus > peripheral lung > brain. Extracts of lung tissues, cultured human bronchial epithelial cells, and fibroblasts had similar alkyltransferase activities. Various human tissues exhibit 2- to 10-fold higher alkyltransferase activity than corresponding rat tissues. The alkyltransferase proteins were prepared as described by Pegg et al. (16). Cell pellets from a variety of human tissues were found to contain activity in normal human tissues and cells (9, 14, 16). We have investigated alkyltransferase activity in various human tissues and compared it to the corresponding rat tissues. The alkyltransferase activities of cultured normal human bronchial epithelial cells and fibroblasts were also compared.

MATERIALS AND METHODS

Materials. The \textsuperscript{3}H-methylated DNA substrate was prepared by reaction of calf thymus DNA with N-[methyl-\textsuperscript{3}H]-N-nitosourea (1.6 Ci/mmol) purchased from New England Nuclear, Boston, MA, as described by Pegg et al. (17). The synthetic DNA substrate containing O\textsuperscript{6}-methyl-[\textsuperscript{8-3}H]guanine was a generous gift from Dr. S. Mitra, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN. Other biochemical reagents were obtained from the Sigma Chemical Co., St. Louis, MO.

Tissues and Cells. Human tissues were collected at the time of surgery and immediate autopsy, transported to the laboratory in L-15 medium at 4°, and frozen at −70° until preparation of extracts (5). Male CD rats (200 to 250 g) were killed by CO\textsubscript{2} asphyxia, and the tracheas, esophagus, and colons were excised and frozen at −70° until extracts were prepared. Human bronchial epithelial cells and fibroblasts were derived and cultured as described by Lechner et al. (10). Chinese hamster V79 cells were grown as described by Hsu et al. (8).

Preparation and Assay of Tissue Extracts. Tissue samples were homogenized in 50 mm Tris-HCl (pH 7.5)-1 mm dithiothreitol-0.1 mm EDTA, and crude extracts containing the O\textsuperscript{6}-alkylguanine-DNA alkyltransferase were prepared as described by Pegg et al. (16). Cell pellets were suspended in the same buffer and broken by sonication (3 times; 30-sec bursts). The extract was centrifuged at 12,000 x g for 10 min, and the supernatant was removed and used as a source of enzyme. The activity was assayed routinely by following the loss of labeled O\textsuperscript{6}-mGua\textsuperscript{a} from \textsuperscript{3}H-methylated DNA using specific antibodies for O\textsuperscript{6}-mGua to isolate this product as described in detail in Ref. 17. The standard assay conditions contained 1.8 pmol of O\textsuperscript{6}-mGua in 1 mg of DNA; 66 mm Tris-HCl, pH 8.3; 1.3 mm dithiothreitol; 0.1 mm EDTA; and up to 15 mg of protein in a total volume to 3 ml. In some experiments, the production of labeled S-methylcysteine in protein was measured as described by Pegg et al. (17). In other experiments, a synthetic DNA substrate containing O\textsuperscript{6}-mGua labeled in position 8 was used to demonstrate the conversion of this product to guanine (17).

RESULTS

Extracts from a variety of human tissues were found to contain O\textsuperscript{6}-alkylguanine-DNA alkyltransferase activity (Chart 1; Table 1). This activity was characterized and quantitated in 3 ways (Chart 1): (a) measuring the specific loss of labeled O\textsuperscript{6}-mGua from a \textsuperscript{3}H-methylated DNA substrate; (b) measuring the production of protein containing S-\textsuperscript{3}Hmethylcysteine during the reaction with

\textsuperscript{a} The abbreviation used is: O\textsuperscript{6}-mGua, O\textsuperscript{6}-methylguanine.
this DNA substrate; and (c) measuring the formation of [8-3H]-
guanine in DNA when the extracts were incubated with a syn-
thetic DNA substrate containing O6-mGua labeled in position 8. 
Increasing amounts of protein of liver, colon, and lung extracts 
gave proportional increases in transalkylase activity until more 
than 1.2 pmol of O6-mGua (corresponding to about 65% of the 
input) were removed. The loss of O6-mGua occurred in parallel 
with a stoichiometric formation of S-methylcysteine in protein. 
With the synthetic DNA substrate, the loss of O6-mGua was 
accompanied by a stoichiometric generation of guanine. Aliquots 
of selected samples were also assayed by measurement of the 
methylated purine content after separation by high-performance 
liquid chromatography. Both methods gave the same results for 
O6-mGua content, and the high-performance liquid chromato-
graphic analysis indicated that 7-methylguanine was not released 
from the DNA substrate.

The specific activity of specimens from several human tissues 
was measured under linear conditions and compared with the 
activity in corresponding rat tissues (Table 1). Extracts of human 
colon, esophagus, and lung had lower activities than those found 
previously in human liver samples but showed somewhat higher 
activities than in human brain. When compared to the corre-
sponding rat tissue, human tissue samples contained 2- to 10-
fold higher levels of alkyltransferase activity.

As can be seen in Chart 2, all 10 human colon specimens and 
12 of 13 human lung specimens showed higher alkyltransferase 
activity than the mean value obtained in the corresponding rat 
organs (Table 1). The interindividual variation of activity on both 
the lung and colon samples was about 4- to 5-fold and showed 
a unimodal distribution.

We also compared O6-alkylguanine-DNA alkyltransferase 
activity in 2 types of cultured human bronchial cells. The removal 
of O6-mGua was proportional to the protein content of the 
extracts in both cell types with up to 2 mg of protein added. 
When assayed under linear conditions, human fibroblasts and 
epithelial cells removed 193 ± 45 and 137 ± 28 fmol O6-mGua 
per mg of protein (S.D.), respectively. No significant difference 
was observed between either exponentially growing or highly 
confluent bronchial fibroblasts. When the activity of Chinese 
hamster V79 cells was assayed under similar conditions as the 
human cells, the activity of V79 cells was so low that it could 
only just be detected in extracts obtained from 106 cells and 
corresponded to 1.6 ± 1.2 fmol of O6-mGua removed per mg of 
protein.

DISCUSSION

Several types of human tissues and bronchial epithelial cells 
and fibroblasts exhibited substantial O6-alkylguanine-DNA alkyl-
transferase activity when crude extracts were isolated and tested 
in vitro with methylated DNA as a substrate. By using 3 different 
methods, the removal of the O6-methyl group from DNA was 
correlated with the formation of S-methylcysteine in protein and 
the regeneration of guanine in DNA. At all protein concentrations, 
there was good agreement between the 3 assays, establishing 
that the reaction does take place by stoichiometric transfer of 
the methyl group from the O6-position of guanine to a cysteine 
acceptor site on the protein. Furthermore, assay of the methyl-
ated purine content of the DNA substrate indicated that the 
extracts did not contain significant nonspecific nuclease activity 
which could contribute to the loss of O6-mGua.

A comparison of the alkyltransferase activity of the different 
human tissues with the corresponding rat tissues indicated that 
human tissues have much higher activities. Human liver was 
previously shown to be 10-fold more active than rat liver (16) 
and all of the human colon, lung, and esophagus samples except 
one showed higher activity than the mean value of the corre-
sponding rat tissues (cf. Table 1; Chart 2). Whereas each tissue 
from individual inbred rats showed a typical experimental vari-
ation that was less than 20%, the human colon (10 cases) or lung 
samples (13 cases) showed a 400 to 500% interindividual vari-

![Chart 1. Effect of protein concentration on O6-alkylguanine-DNA alkyltransferase activity. Results are shown for the loss of O6-mGua from the DNA substrate (\( \Theta, \bigodot, \bigcirc \)), production of S-methylcysteine in the protein (\( \Theta, \bigodot, \bigcirc \)), and production of guanine in the substrate (\( \bigodot, \Delta, \triangle \)). Protein from livers, colon, and lung was tested as shown.](chart1.png)

![Chart 2. O6-Alkylguanine-DNA alkyltransferase activity of individual human colon and lung specimen. The activities of individual specimens of colon (A) or lung (B) were assayed as described in "Materials and Methods." The mean values and the range of activities are presented in Chart 1.](chart2.png)
In a recent study, Krokan et al. (9) have shown a less than 10-fold interindividual variation of alkyltransferase activity in extracts prepared from liver, colon, and stomach. However, a 42-fold interindividual variation was observed in 12 specimens from small intestine. Several human fetal tissues contain similar levels of alkyltransferase activity as adult tissues, except that 5-fold lower levels were observed in fetal than in adult liver (9). The alkyltransferase activity of extracts from human lymphocytes has been shown to vary approximately 8-fold (19, 20), and human lymphocytes vary considerably in their capacity to remove O\textsuperscript{6}-mGua (7). However, the levels of this DNA repair activity in human tissues and cells do not vary among individuals as much as the activities of enzymes responsible for metabolic activation and deactivation of chemical carcinogens (6).

Since the O\textsuperscript{6}-alkylguanine-DNA alkyltransferase is consumed and inactivated by accepting the methyl group from the DNA substrate, the number of acceptor sites present in the tissues or cells can be estimated. Assuming that only one methyl group was bound per molecule, and that calculation was on the basis of DNA content, human liver contains approximately 500,000 alkyltransferase molecules per cell, whereas colon, esophagus, and peripheral lung contain 76,000, 65,000, and 39,000 enzyme molecules, respectively. Isolated cultures of human bronchial fibroblasts and human bronchial epithelial cells both contained the activity. Although the fibroblasts were slightly more active in this respect on a per protein basis, the 2 cell types have similar activities on a per cell basis, i.e., 26,000 (epithelial cells) and 29,000 (fibroblasts) alkyltransferase molecules/cell. Previous estimates of alkyltransferase activity in cultured human cells similarly indicate high levels in skin fibroblasts (13), while different human tumor cell lines range from nondetectable levels to 2 \times 10\textsuperscript{5} alkyltransferase molecules/cell (4, 23). The activity of the bronchial cells is about one-half of the activity present in freshly isolated rat hepatocytes (18) but is very much greater than in cultured V79 hamster lung fibroblasts which contained very little activity. The activity of V79 cells was so low that it was barely detectable in extracts obtained from large numbers of cells and amounts to only 200 molecules/cell. Although Waldstein et al. (21) found 94,000 molecules/cell in their cultures of V79 cells, results from other studies agree with the low activity of O\textsuperscript{6}-alkylguanine-DNA alkyltransferase reported here (2, 4, 14, 23).

In conclusion, several human tissue and cell types demonstrate a capacity to repair O\textsuperscript{6}-mGua lesions in DNA. For all tissues compared, this capacity is generally several-fold higher than in rats, in which N-nitroso compounds cause the formation of O\textsuperscript{6}-alkylguanine lesions in cellular DNA and tumors in many organs (12). Further studies are required to elucidate the relationship, if any, of the comparatively high levels of O\textsuperscript{6}-alkylguanine-DNA alkyltransferase activity in normal human tissues and cells and oncogenic susceptibility to N-nitroso carcinogens in humans.

ACKNOWLEDGMENTS

We appreciate the helpful comments of R. Bennet and H. Krokan. The technical assistance of L. West and W. Pettis is also appreciated.
$O^6$-Alkylguanine-DNA Alkyltransferase Activity in Normal Human Tissues and Cells

Roland C. Grafstrom, Anthony E. Pegg, Benjamin F. Trump, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/7/2855

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.