Immunohistochemically Detected O6- and N7-Methyldeoxyguanosine in Organs of Rats Administred N-Methyl-N-Nitrosourea

Mitsuaki Kitano¹, Takashi Murai¹, Masao Shizusawa¹, Seiko Tamano², Akihiro Hagiwara², and Shoji Fukushima¹

First Department of Pathology, Osaka City University Medical School, and ¹Daiyu-kai Institute of Medical Science

Abstract: The production of DNA adducts, O6-methyldeoxyguanosine (O6-medG) and N7-methyldeoxyguanosine (N7-medG), was examined in various organs of rats administered N-methyl-N-nitrosourea (MNU) using an immunohistochemical approach. Fisher 344 rats divided into 4 groups were treated with alkylating agents. Animals in groups 1 and 2 were administered MNU (20 mg/kg) intragastrically and intraperitoneally, respectively. Animals in group 3 were administered dimethylnitrosamine (DMN) (60 mg/kg, i.p.) as a positive control and group 4 was maintained as a negative control group. At 10 hours after the carcinogen administration, rats were killed under ether anesthesia and the liver, stomach, and esophagus were removed from each. In the animals treated with MNU i.g., N7-medG positive nuclei were found in forestomach, glandular stomach, liver, and esophagus while only the liver and forestomach were positive after the i.p. treatment. O6-medG positive cells were not detected in any organs of animals receiving MNU (i.g. or i.p.). In the livers of animals treated with DMN, nuclei of hepatocytes were strongly stained with antibodies to both O6- and N7-medG. In the control group no O6- or N7-medG positive cells were detected in any of the organs. (J Toxicol Pathol 1997; 10: 73–75)

Key words: O6-methyldeoxyguanosine, N7-methyldeoxyguanosine, N-methyl-N-nitrosourea, Rat

Introduction

Alkylating agents form DNA adducts in the nuclei of target cells, the final result often being cancer induction. O6-methyldeoxyguanosine (O6-medG) and N7-methyldeoxyguanosine (N7-medG) are well known DNA adducts caused by methylating agents such as N-methylnitrosourea (MNU), and N-methyl-N'-nitro-N-nitrosoguanidine. The predominant DNA adduct generated in such cases is N7-medG accounting for more than 70 per cent of the total. Although the O6-medG amount is only about 10 per cent of DNA adducts, it leads to point mutations in DNA following replication. However, assays of N7-medG increase sensitivity allowing measurement of low level exposure. Examination of DNA adduct formation in target and non-target organs is useful to cast light on the relationship between DNA damage of different types and carcinogenesis.

N-nitroso compounds are alkylating agents, which constitute one of most important risk factors for neoplasia in man. In the present study MNU and dimethylnitrosamine (DMN) were chosen as representative examples. MNU is a direct acting carcinogen which does not require metabolism and causes tumors in many organs. The degree of carcinogenicity in the different target organs, however, depends on the route of administration. The i.g. and i.p. routes were chosen for examination here as those most often applied. DMN is a hepatocarcinogenic agent well known to cause O6-medG and N7-medG adduct formation after metabolic activation in the liver target organ. An immunohistochemical technique was employed for direct visualization of adducts in tissue sections.

Materials and Methods

Animals

Eight male 7-week-old F344 rats were purchased from Charles River Japan Inc., Hino, Shiga. They were housed in an animal room maintained with a 12 hr (7:00-19:00) light-dark cycle, at a constant temperature of 25±1°C, and a relative humidity of 55±5%. The animals were observed daily, and were used after a 1-week acclimation period for the experiment detailed below.

Chemicals

MNU was purchased from Wako Pure Chemical Ind. Ltd. (Osaka). DMN was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo).

Treatment

Eight male 8-week-old F344 rats were allocated into 4 groups of two animals each. Groups 1 and 2 were given MNU (20 mg/kg body wt) intragastrically or intraperitoneally, respectively. Animals in group 3 were administered DMN (60 mg/kg body wt., single i.p. injection) as a positive control. The negative control group (group 4) received powdered basal diet only without any treatment. At hour ten after the carcinogen administration, rats were killed under ether anesthesia. The liver, forestomach, glandular stomach, and esophagus were removed from each rat, and tissue slices were fixed in cold acetone for 2 weeks. Then they were routinely processed for embedding in paraffin, and sections were cut at 4 μm.
Detection of O6- and N7-medG in Rats

Table 1. Immunohistologic Finding for Digestive Organs of Rats with Anti-O6-medG and N7-medG Antibodies

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Foremastomach</th>
<th>Glandular Stomach</th>
<th>Esophagus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O6-medG</td>
<td>N7-medG</td>
<td>O6-medG</td>
<td>N7-medG</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

# strongly positive, + moderately positive, + slightly positive, – negative
NE; not examined

Immunohistochemistry

The preparation and specificity of the monoclonal O6-medG and polyclonal N7-medG antibodies used were described previously. After deparaffinization, sections were sequentially treated with 0.05N sodium hydroxide in 40% ethanol for 5 min, 5% acetic acid in 40% ethanol for 15s and 1% non-fat milk in phosphate-buffered saline for 1h, and then exposed to primary antibodies against O6-medG (1:1, 250) and N7-medG (1:2,500) at 4°C overnight. As a negative control, we used normal rabbit serum instead of the first antibody. Demonstration of antibody binding was carried out using the avidin-biotin complex immunohistochemical method. The tissue sections were lightly counterstained with hematoxylin to facilitate orientation on histopathological examination.

Results and Discussion

Results for staining intensity of nuclei in the liver, esophagus, forestomach and glandular stomach are summarized in Table 1. In the animals treated with MNU intragastrically (group 1), nuclei strongly positive for N7-medG were found in the squamous epithelium of the forestomach and the surface epithelium of the glandular stomach (Fig. 1-a, b). Furthermore, nuclei of hepatocytes in the liver and squamous epithelial cells in the esophagus were moderately stained with antibodies against N7-medG (Fig. 1-c). This is of interest because limited DNA adduct formation in the esophagus by MNU has been reported. In the animals treated with MNU intraperitoneally (group 2), hepatocytes in the liver and squamous epithelial cells in the forestomach were stained.
Fig 2. Immunohistochemical detection of O6-medG (a) and N7-medG (b) positive cells in the liver of a rat administered DMN (60 mg/kg, i.p.). a, b: ×65

With antibody to N7-medG moderately or slightly, but not the other organs. The results for liver are in agreement with a weak carcinogenic action of MNU in this organ. However O6-medG positive cells were not detected in any tissues in groups 1 and 2. In the livers of animals treated with DMN intraperitoneally (group 3), in contrast, hepatocytes were strongly stained with both antibodies to O6-medG and N7-medG. This was especially the case in the centrilobular zone whereas hepatocytes around the portal triads demonstrated weak O6-medG and moderate N7-medG staining (Fig 2). The present finding of stronger binding in centrilobular hepatocytes (zone 3) is in line with earlier reports. In the control group no O6- and N7-medG positive cells were detected in any organs.

The present results thus indicated that MNU administration by the intragastric route causes aducts in more of the digestive tract than when given i.p. The fact of no O6-medG positive cells presumably reflects low initial formation and rapid repair of O6-medG than N7-medG adducts. Whether O6-medG adducts were present early or at a level below the detection limit of immunohistochemistry remains unclear.

Acknowledgment: We would like to express our gratitude to Professor Christopher P. Wild (Molecular Epidemiology Unit, Research School of Medicine, University of Leeds) and Dr. Hiroyuki Tsuda (Chemotherapy Division, National Cancer Research Institute) for provision of antibodies.

References