Evaluation of methyl methacrylate monomer cytotoxicity in dental lab technicians using buccal micronucleus cytome assay

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Methyl methacrylate (MMA) monomer, a primary component of dental resins, is known to induce cytotoxicity, dermatitis, and neuropathy. The objective of this study was to assess the incidence of micronuclei (MN) in buccal mucosal cells of dental technicians exposed to MMA using Buccal Micronucleus Cytome (BMCyt) assay. The Risk Group (RG=13) consisted of all the technicians working in the prosthetic production laboratory of KKU-College of Dentistry. The Control Group (CG=14) consisted of healthy students and doctors matching the age of RG subjects. Buccal mucosa scrapes obtained from all the 27 RG and CG subjects were stained with Papanicolaou stain and observed under oil immersion lens (100×) for the presence of MN. There were no significant differences in the incidence of MN between RG and CG (p>0.05).

Keywords: Micronucleus, Buccal epithelial cells, Dental technicians, Methyl methacrylate

INTRODUCTION

Methyl methacrylate (MMA) monomer, a colorless strong-smelling liquid, is extensively used in a wide range of dental and medical applications. It is a principal ingredient of many dental restorative and prosthetic resins. It is also frequently used as a bone cement in neurosurgical and orthopedic surgical procedures to fill spaces in bones.

Concerns regarding the toxicity of MMA have been raised since the early 1940s. In an in vitro study in which leukocytes and endothelial cells suspended in culture medium were exposed to MMA, the latter showed marked signs of cytotoxicity. The clinical implication gleaned from this study was that MMA could induce an inflammatory response in leukocytes and endothelial cells and cause deep vein thrombosis. In the oral cavity, MMA in dental resin composites could induce DNA double-strand breaks in primary human oral cells with severe phenotypic consequences. In MMA-fixed hip and knee joint replacement surgery, direct implantation of MMA in the human body could lead to cardiorespiratory collapse and death.

In the dental laboratory where dental technicians (DTs) make dental prostheses, they are occupationally exposed to MMA. Regular contact with and chronic inhalation of MMA has caused toxic side effects, ranging from allergic contact dermatitis, stomatitis, paraesthesia of the finger tips to neuropathy, liver toxicity, hemorrhage, and necrosis of lung tissue. Interestingly, prolonged inhalation of MMA did not increase the carcinogenic effect in mice.

Toxic exposure causes small extra nuclear bodies called micronuclei (MN) to form at anaphase during the nuclear division phase of cell cycle. MN are small nuclei separate from and additional to the main nuclei of cells, and they contain chromosomal fragments or whole chromosomes. MN are one of the established genotoxicity biomarkers in human erythrocytes, lymphocytes, reticulocytes, and buccal mucosa cells. An increased frequency of MN is used as a measure to detect clastogenicity and aneugenicity.

Buccal mucosa cells are very sensitive to toxicity. As they readily form MN in response to toxic exposure, buccal mucosa cells are used as a source of tissue for monitoring human exposure to toxic substances encountered in occupational and environmental settings. Due to the ease of sampling and collecting buccal mucosa cells, Buccal Micronucleus Cytome (BMCyt) assay has been widely used since the 1980s to screen groups that are exposed to or have inhaled or ingested toxic pollutants. This simple, sensitive, and reliable method involves two key steps to calculate MN formation frequency: stain exfoliated buccal epithelial cells collected from the inner wall of the cheek, and then observe the smear under a light microscope.

To date, a database search of published literature showed that no studies have been carried out to investigate the effect of MMA on buccal mucosa cells. Therefore, the aim of this study was to evaluate the effect of MMA on buccal mucosa cells using BMCyt.

MATERIALS AND METHODS

Human subjects
The Risk Group (RG) consisted of all the DTs working in the prosthetic production laboratory, KKU-College of Dentistry, for at least one year before the start of this study (n=13). The Control Group (CG) consisted of 14 students and doctors matching the age of RG subjects (Table 1). CG subjects were not using any form of mouth
Table 1  Distribution of human subjects in Control and Risk Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Students</th>
<th>Doctors</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>Male</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>RG</td>
<td>Male</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>13</td>
</tr>
</tbody>
</table>

Table 2  Levels of incidence of micronuclei in RG and CG

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Median</th>
<th>Std. Deviation</th>
<th>Std. Error of the mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG</td>
<td>13</td>
<td>5.21</td>
<td>7</td>
<td>2.20</td>
<td>0.61</td>
</tr>
<tr>
<td>CG</td>
<td>14</td>
<td>6.23</td>
<td>5</td>
<td>2.42</td>
<td>0.65</td>
</tr>
</tbody>
</table>

RESULTS

Table 2 details the mean, median, standard deviation, and standard error results of the statistical analysis. The t-test was used to compare the means of RG and CG.

In RG, the mean frequency (or number) of MN in a total of 2000 cells was 6.23; in CG, it was 5.21. Difference between the mean MN in dental lab technicians (RG) and general population (CG) was 1.02, with a 95% confidence interval from –0.82 to 2.86. The t-test statistic was 1.1371, with 25 degrees of freedom and an associated p-value of 0.2663. Therefore, the incidence of MN cells in RG was not significantly different from that in CG.

DISCUSSION

Monomers released from dental resins can cause adverse biological effects in mammalian cells. MN formation is indicative of chromosomal damage, and a vast number of studies have shown that resin monomers like triethyleneglycol dimethacrylate (TEGDMA) and 2-hydroxyethyl methacrylate (HEMA) could cause gene mutations and even alter the functions of the cells of the oral cavity13).

MMA has been shown to induce DNA double-strand breaks, and MMA toxicity is a longstanding health hazard concern for DTs. They often handle MMA bare-handed before the polymerization reaction, and the monomer can penetrate the skin during the process14). Prolonged cutaneous absorption can result in local neurotoxicity, where mild axonal degeneration was revealed in areas with the closest and most frequent contact with MMA 14). MMA can also be inhaled or ingested, which may cause local mucosal irritation or even an allergic reaction. In a clinical case of a DT who had 30 years of occupational cutaneous and inhalational exposure to MMA, neurophysiological test showed that he had developed generalized sensorimotor neuropathy of axonal degeneration type with loss of large myelinated fibers and unmyelinated axons15).

For working populations exposed to occupational inhalants, they are in high risk of developing cytogenetic disorders. Increased MN frequency in the buccal mucosa cells of fire fighters after prolonged exposure to combustion fumes unveiled their heightened susceptibility to cancer16). Similarly, increased MN frequency detected in petrol station attendants17) and calcite factory workers18) put them under significant risk of developing several types of cancer. Individuals that should periodically undergo biological monitoring and proper care include hairdressers19), car battery repair workers and car painters20) because of their daily exposure to harmful chemicals.

In the present study, the formation frequency of MN in RG when compared to CG was highly insignificant (p=0.27). This could in part be due to personal protection methods rigorously practiced by
DTs when handling MMA, such as wearing face masks and hand gloves. On workplace safety, modern prosthetic production laboratories are well equipped with facilities and devices, such as sufficient exhaust ventilation, to minimize the risk of MMA toxicity in DTs.

CONCLUSIONS
Occupational exposure to MMA did not lead to statistically higher incidence of MN in the buccal mucosa cells of DTs. Therefore, it is highly recommended to continue using face masks and hand gloves in a sufficiently ventilated room when handling MMA. In view of the few studies conducted on DTs, it would be interesting to pursue a similar study on nasal mucosa cells with the same study groups.

REFERENCES
1) Deichmann W. Toxicity of methyl, ethyl, and n-butyl methacrylate. J Ind Hyg Toxicol 1941; 23: 343-351.