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Cytotoxic and genotoxic effects of resin monomers in human salivary gland tissue and lymphocytes as assessed by the single cell microgel electrophoresis (Comet) assay

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Abstract

Malignant tumors of the three major pairs and the numerous minor salivary glands in humans are rare, and little is known about their various etiologies. Considering the fact that resin monomers from dental restorative materials are released into the saliva and diffuse into the tooth pulp or gingiva, mucosa, and salivary glands, this may potentially contribute to tumorigenesis. Resin monomers may also be reabsorbed and reach the circulating blood as well. Whereas the cytotoxic potential of some components has been clearly documented, data on genotoxicity in human target cells require further investigation. In the present study, genotoxic and cytotoxic effects of three common methacrylates are investigated in human samples of salivary glands and peripheral lymphocytes.

The Comet assay was used to quantify DNA single strand breaks, alkali labile and incomplete excision repair sites in salivary gland probes and lymphocytes of 10 volunteers. The xenobiotics investigated were triethyleneglycoldimethacrylate (TEGDMA), urethanedimethacrylate (UDMA), and 2-hydroxyethylmethacrylate (HEMA), with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) and dimethyl sulfoxide (DMSO) as controls. DNA migration was analyzed using the tail moment according to Olive (OTM). Cytotoxicity was monitored using trypan blue staining.

With TEGDMA concentrations at 10^{-5} M (10^{-3} M), UDMA at 10^{-7} M (10^{-7} M), and HEMA at 10^{-3} M (10^{-5} M) significant enhancements of DNA migration were achieved in tissue cells (lymphocytes) as compared to the negative controls. At higher concentrations of up to 2.5×10^{-2} M, induced DNA migration was expressed by OTM at 10.7 for TEGDMA in tissue cells (8.7 in lymphocytes), 10.5 for UDMA (6.4), and 9.7 for HEMA (6.1). The viability of the cell systems was not affected as concerns the threshold level for the assay of 75% viable cells except for the highest concentration tested for TEGDMA and UDMA in tissue cells.

At higher concentration levels, all tested substances induced significant enhancement of DNA migration in the Comet assay as a possible sign for genotoxic effects in human salivary glands and lymphocytes. These data add to the results of prior studies in human peripheral lymphocytes and give evidence of a possible risk factor for tumor initiation in human salivary glands.

Keywords: Comet assay; Resin monomers; Tumorigenesis; Human cells; Salivary glands; Lymphocytes

1. Introduction

Based on preliminary reports suggesting genotoxic effects of dental restorative materials, such as triethyleneglycoldimethacrylate (TEGDMA), urethanedimethacrylate

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(UDMA), and 2-hydroxyethylmethacrylate (HEMA) in human peripheral lymphocytes [1], further studies were warranted in target cells located in immediate proximity to the teeth. In the present study, human salivary gland tissue cells were investigated since a variety of neoplasms may develop in these glands. Whereas in the large parotid glands the majority of tumors is benign, in the small glands, located in the oral and pharyngeal as well as laryngeal mucosa, malignomas prevail. In contrast to mucosal squamous cell carcinomas of the oral cavity, pharynx and larynx, where the predominant risk factors are tobacco smoke and alcohol consumption, the etiology of salivary gland tumors remains unclear. Environmental factors such as radiation, smoking status, profession and xenobiotics have been discussed as potential hazards [e.g., 2,3].

In replacing amalgam, resin composites and glass ionomer cements are used to restore cavities in the primary and permanent dentition with tooth-colored materials and a variety of substances is used for the bonding process. However, some components of the composites and bonding materials may become segregated in an aqueous environment during implantation and even after polymerization [4–6]. Here they may exert adverse effects on the organism, e.g., allergic reactions such as urticaria and contact dermatitis [7], systemic toxicity, cytotoxicity, estrogenicity, and mutagenicity [8,9].

TEGDMA and HEMA are commonly used as co-monomers in resin composites and dental bonding to influence viscosity and bonding strength of composites comprising, e.g., UDMA [10]. Their original contents vary from 25% to 55%, but they are partially released in an aqueous phase [11] and diffuse through the dentine into the pulp space [12]. Released monomers may either be taken up by the mucosa of the mouth or pharynx or may be swallowed via the saliva and excreted with the urine [13]. Therefore, two potential routes how the monomers may effect the salivary gland tissues are possible: first, direct contact with the mucosa that contains an abundance of small salivary glands; second, uptake into the circulating blood and a possible excretion via the salivary glands. Thus, it appears appropriate to monitor these substances for genotoxicity, especially since there is evidence for xenobiotics, such as asbestos in the larynx and pharynx [14], wood dust [15] and chromium [16] in the nose to add to a tumor risk in the head and neck region. In the case of wood dust, specific tumor entities are induced [17], but there is no such evidence for xenobiotics and salivary gland tumors. However, salivary gland tumors may still be related to the exposure to xenobiotics since there are data suggesting, e.g., genotoxic effects on human salivary gland tissue by pesticides and metals [18] and metals and ethanol [3].

The alkaline single cell microgel electrophoresis (Comet) assay detects genotoxicity in a wide variety of human cell materials [19,20], and has proven to be a sensitive and valid in vitro method as well [21–25]. The purpose of the present investigation was to assess the genotoxic potential of TEGDMA, HEMA, and UDMA in macroscopically healthy human salivary gland tissue and peripheral lymphocytes in vitro.

2. Materials and methods

2.1. Donors

The ethics committee of the University of Regensburg Medical Department approved this study on parotid gland and blood specimens of 10 patients. There were nine male and one female donors (Table 1). Whereas the access to lymphocytes is relatively safe by venous puncture and venous catheters, the harvest of salivary gland tissue is limited to surgery of the glands. Such surgical procedures are most commonly performed for benign salivary gland tumors and entail the risk of damage to the facial nerve. Therefore, the samples for this study were gathered from patients with benign parotid gland tumors, where surgery had included the removal of the lateral part of the parotid gland including larger portions of non-tumor tissue, the latter being used for this study. All donors were otherwise healthy. They had consented in written form. The Declaration of Helsinki was obeyed.

2.2. Cell preparation

Biopsies of the parotid glands were taken during parotid surgery and placed in MEM-Joklik (without L-glutamine and $NaHCO_3$; Linaris, Bettingen, Germany), and transferred to the laboratory immediately after collection. The samples were digested enzymatically by treatment with collagenase P (1 mg/ml) (Boehringer, Mannheim, Germany), hyaluronidase

Table 1
Characteristics of patients

Patient	Age [a]	Gender	Profession	Nicotine [py]	Alcohol [g/d]
1	57	m	Mechanic	35	0
2	66	m	Pensioner	40	200
3	54	m	Mechanic	30	100
4	58	m	Carpenter, pensioner	0	400
5	66	m	Farmer	20	0
6	83	m	Butcher	80	120
7	41	f	Nurse	40	20
8	54	m	Butcher	0	0
9	73	m	Construction worker, pensioner	0	0
10	55	m	Technician	15	0

a = years; m = male; f = female; py = package year (365 day × 20 cigarettes/day); g/d = grams per day.