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An In Vivo Investigation into Oxidative Stress of Methacrylate Monomers Used In Dentistry

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ABSTRACT

The role of glutathione depletion and ROS formation as a major contributor to the cytotoxic effect of methacrylate monomers used in dentistry. We aimed to evaluate the oxidative stress induced by methyl methacrylate *in vivo*. ***Methods:*** Twenty five subjects, 25 dental handle and 25 healthy controls were included in the study. Depletion of glutathione (GSH), *glutathione peroxidase* (GPx), *glutathione-s-transferase* (GST) and the total antioxidant status (TAS) was evaluated from blood samples. ***Results:*** Patients and workplaces showed significant decrease in serum antioxidant biomarkers levels ($p \leq 0.05$). There was a significant decrease of patients GSH levels with increasing MMA contact frequency, increasing number of teeth with temporary prosthesis and longer duration of prosthesis wearing ($p \leq 0.05$). A significant decrease of workplaces GSH levels was observed with longer duration of exposure to MMA ($p \leq 0.05$). ***Conclusions:*** Our findings suggest that increase in ROS caused by the sequestering of GSH seems only partly to be responsible for the observed toxicity of MMA. ***Clinical significance***: There is an acceptance that research in vivo should ultimately be of relevance and benefit to patients rather than focus on technical aspects of interventions. This study points to an undue emphasis on oxidative stress induced by methyl methacrylate used in dentistry in vivo, which may explain the possible mechanism of toxicity of these compounds by which they may exert their effect on cells.

INTRODUCTION

Polymethyl methacrylate resins are being used extensively for direct restorations in daily prosthodontic practice. These materials are polymerized in situ, however, the conversion from methyl methacrylate monomers (MMA) to polymer is never complete, therefore some unreacted monomers called residual monomers are left [1-5]. This has been demonstrated both *in vitro* and *in vivo* from materials after setting [6,7,8], and it has been shown that MMA can pass through dentine channels and enter the circulation [9]. In addition, dental personnel handle the uncured materials and are potentially exposed to the monomers on a daily basis. Both direct skin contact and airway exposure to uncured methacrylate occur [10-13].

Several studies have shown that methacrylate-based monomers from dental resin-based materials have the potential to cause adverse effects in mammalian *in vitro* systems [14]. Cytotoxic [15], genotoxic [16-20], and eostrogenic [21] effects have been discussed and demonstrated. Glutathione (GSH) sequestration by adduct formation between cysteine in GSH and the methacrylate monomers, followed by formation of reactive oxygen species (ROS), has been pointed out as a key event in increased oxidative stress in cells thus in observed toxic response [22,23].

GSH is an important antioxidant in the human body and is found in high concentrations in cells in the cytosol, mitochondria, and the cell nucleus. GSH contains the amino acid cysteine, and the antioxidative activity is connected to the thiol group in this amino acid [24]. The role of glutathione depletion and ROS formation as a major contributor to the cytotoxic effect of methacrylate monomers has not been fully elucidated. Hence, this study was carried out with the objective to evaluate the oxidative stress induced by methyl methacrylate in vivo, this information would throw light on the possible mechanism of toxicity of these compounds by which they may exert their effect on cells is of great value for risk evaluation of new and existing dental materials.

MATERIALS AND METHODS

Study population

The cases comprised a total of 25 subjects [12 men and 13 women; age (mean ± SD):45.84± 10.12 years] ascertained from the department of fixed prosthodontics, faculty of dental medicine, Monastir, Tunisia. Dental workplace group included 25 dental handles recruited

from the same department [10 men and 15 women; age (mean ± SD):44.25 ± 11.23 years]. 25 blood donor volunteers recruited from Fattouma Bourguiba Hospital, Monastir, Tunisia, and had a similar age distribution [11 men and 14 women age (mean ± SD): 44.72 ± 10.34 years] were included in this analysis as control group. All study participants showed no disease. The study was reviewed and approved by the local ethics committee and included only individuals that agreed to participate after reading and signing a free and informed consent form.

Interviews

Patients were selected from the clinic of dentistry allocated for the study. An intra-oral examination was conducted and the clinical files were checked to structure the questionnaire. The respondents were asked about their medical and dental history, then the contact frequency with MMA was mentionned as well as the number of teeth with temporary prosthesis and how long they carry the temporary prosthesis. Participants were also examined about the type of restoration, teeth vitality, pulpless teeth and cervical margin in contact with the gingiva.

Laboratory measurements

5ml blood samples from cubital vein were collected. Out of that 2ml was collected in heparinized bulb and the remaining was allowed to clot. Plasma and serum were separated by centrifugation at 3000 rpm for 10 minutes at room temperature and was analyzed. GSH is oxidized by 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) resulting in the formation of GSSG and 5-thio-2-nitrobenzoic acid (TNB). GSSG is then reduced to GSH by *glutathione reductase* using reducing equivalent provided by NADPH. The rate of TNB formation is proportional to the sum of GSH and GSSG present in the sample and is determined by measuring the formation of TNB at 412 nm [25]. *Glutathione peroxidase* (GPx) was measured at 340 nm through the glutathione/ NADPH/ *glutathione reductase* system by the dismutation of cumene hydroperoxide [26]. *Glutathione S-transferase* (GST) activity was determined at 340 nm according to a transfer reaction of GSH on the CNDB (chloro-1, 2, 4 dinitrobenzene) [27]. The oxidation system of ABTS (2,2'-azino-di (3-ethylbenzthiazoline-6-sulfonic acid)) by myoglobin with hydrogen peroxide (H_2O_2) at 600 nm was used to measure total antioxidant status (TAS) [28].

Statistical data analysis

Statistical analysis was done with SPSS software (version 17, Chicago, IL, USA). The data was expressed as mean ± standard deviation and statistical significance was considered for $p \leq 0.05$.

RESULTS

Antioxidant biomarkers in patients with temporary prosthesis, dental workplaces and healthy controls are given in table 1.

Table 1. Antioxidant biomarkers of patient, dental workplace and control groups.

	Groups (Mean ± SD)			
	Patients (n=25)	**Workplaces (n=25)**	**Controls (n=25)**	**P value**
GSH[a] (µmol/L)	4.21±2.5	6.46±3.1	8.72±2.6	0.034
GPx[b] (u/L)	161.41±6.32	187.22±5.86	572.12±7.38	0.03
GST[c] (u/ml)	8.3±2.4	10.3±2.8	12.1±4.6	0.02
TAS[d] (mmol/L)	1.23±0.19	1.37±0.26	1.99±0.78	0.02

GSH[a]: glutathione; GPx[b]: glutathione peroxidase; GST[c]: glutathione s-transferase; TAS[d]: total antioxidant status.

Patients exhibited significant decrease ($p \leq 0.05$) in serum antioxidant biomarkers levels when compared to workplaces and healthy controls. Furthermore, in workplaces, the analysis of antioxidant levels showed a significant decrease in GSH, GPx, GST and TAS activities compared to control group ($p \leq 0.05$).

A statistically significant decrease of patients GSH levels was associated with increased MMA contact frequency, increased number of teeth with temporary prosthesis and longer duration of prosthesis wearing ($p \leq 0.05$). In addition, the results showed a statistically

significant decrease of GSH levels in workplaces associated with longer duration of exposure to MMA ($p \leq 0.05$) (table 2).

Table 2. Distribution of glutathione levels in patients and dental workplaces.

	Patients (n=25)	**Workplaces (n=25)**	**P value**
Contact frequency with MMA[a]			
1	5.71±2.6	-	0.02
2	4.38±2.2	-	0.02
3	2.54±2.8	-	0.03
Number of teeth with temporary prosthesis			
1-2	5.36±3.1	-	0.047
3-5	3.89±2.7	-	0.03
> 5	3.38±2.2	-	0.03
Duration of prosthesis wearing (weeks)			
	5.89±3.4	-	0.02
	4.22±2.1	-	0.025
1-2	2.52±2.6	-	0.043
3-8			
> 9			
Duration of exposure to MMA[a] (months)			
	-	3.86±2.4	0.036
	-	3.24±2.8	0.002
1-12	-	2.88±2.1	0.047
13-24	-	2.65±3.2	0.04
25-48			
>48			

MMA[a] : methyl methacrylate.

DISCUSSION

The present study provides new insights into the mechanism of MMA-induced toxicity either in patients and dental workplaces. Our results showed that MMA-induced toxicity is associated with decreased levels of antioxidant biomarkers, in particular, GSH, GPx, GST in patients and dental workplaces compared to controls. These results are in broad agreement with the recent *in-vitro* report published by Morisbak et al. [24] in which authors founded that methacrylate monomers induces cell proliferation disturbances and cell toxicity through glutathione depletion and subsequent ROS formation.

Antioxidants by counteracting the harmful effect of free radicals protect structural and tissue integrity. Imbalances between free radicals and antioxidants have been suggested to play an important role in the onset and development of toxicity. Antioxidant enzymes like GPx and GST provide protection against oxidative injury from oxygen free radicals. The function of GPx is to reduce hydrogen peroxide and/ or lipid hydrogen peroxides by the oxidation of reduced glutathione or s-nitroso glutathione, whereas GST comprises a group of enzymes that are also able to detoxify a variety of compounds including xenobiotics derived from pathogenic microorganisms, catalyzing their conjugation with GSH [29]. The GPx increase was reported as indirect marker of oxidative stress and may represent possible antioxidant compensation in detoxification reactions of organic peroxides produced during oxidative stress [30].

In this study, total antioxidant status is significantly diminished in patients compared to healthy controls. The total antioxidative potential of the plasma reflects the ability of an individual to resist the oxidative stress. Furthermore, TAS has the advantage that it analyzes the combined effectiveness of contributing species, which may be greater than the sum of the individual antioxidant.

Our results, in line with numerous studies which focused on the toxicity of MMA monomers in dental workplaces [31,32,33], revealed a significant decrease in serum antioxidant biomarkers levels. Since, several body systems appear to be affected, including the skin, the respiratory tract, and the neurological system. Dentists and other dental staff who work with this material may be exposed when they occasionally contact MMA monomer directly, such as when relining a denture or making a temporary crown. The preparation of dental

prostheses and orthodontic appliances by dental technicians and other dental staff also involves manual handling and dermal exposure to MMA may occur [31].

It has been shown that MMA affects the integrity of latex examination gloves that are used in dental clinics, which allows MMA to penetrate the skin [32]. A Finnish study of 163 dental technicians and technical assistants, who reported daily dermal contact with MMA-containing compounds, found that only three subjects wore protective gloves during acrylic molding and only 15 subjects wore gloves while performing other tasks that may have involved exposure to MMA [33].

It has previously been found that methacrylate monomers induce glutathione depletion by adduct formation [22,34]. The glutathione depletion is assumed to be the cause of the reported ROS increase in cells exposed to methacrylate [22]. Increased intracellular ROS is potentially toxic to cells, and we wanted to found a correlation between exposure to MMA and in vivo glutathione depletion. In this study, we found that MMA-induced toxicity is associated with a rapid depletion of GSH, in agreement with several previous study performed with eluates of resin-containing dental restorative materials [22,24,34,35]. In addition, the data provide the first in vivo evidence that MMA-induced GSH depletion is associated with the subsequent production of reactive oxygen species (ROS). The electron deficient beta carbon of the carbon double bond in methacrylates should be able to react by addition to nucleophilic sites. A mechanism involving complex formation between MMA and the thiol group of GSH may thus be responsible for the observed GSH depletion. Glutathione is normally involved in reactions that oppose the continuous production of ROS by mitochondria. Sequestration of GSH by methacrylate monomers could impair this defense and ultimately result in mitochondrial damage. On the basis of these findings, we suggest a reaction between MMA and the cysteine residue of glutathione as shown in Figure 1.

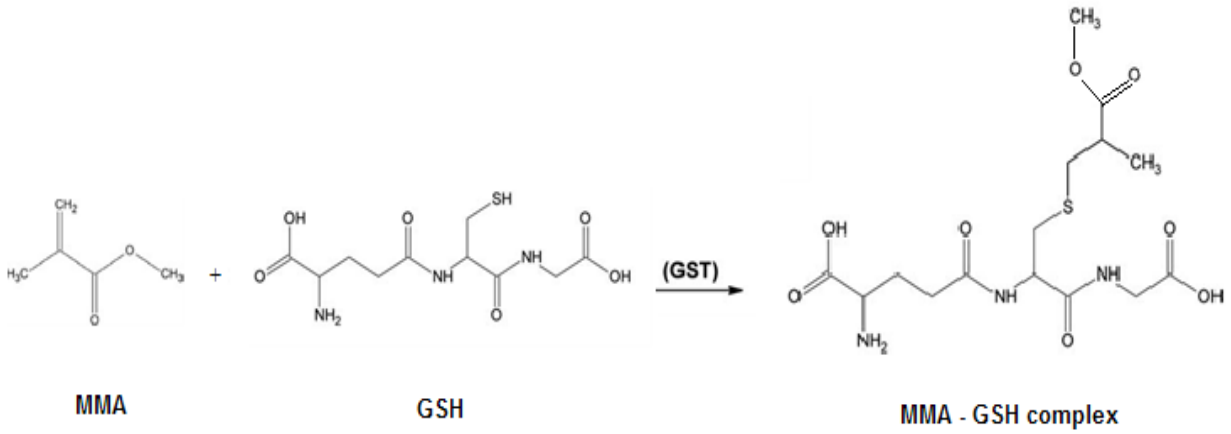

Figure 1: Suggested reaction for complex formation between methyl methacrylate and glutathione.

Our results suggest that MMA has the ability to bind to cysteine residues which suggest that MMA has the ability to covalently bind groups on cellular macromolecules. Such binding could lead to altered enzyme activity, which may be important in the initiation of toxic effects. Although the formation of DNA adducts has not been reported, this is another interesting possibility and should not be excluded.

DNA damage after exposure to methacrylate monomers is observed in several studies [36-38]. In most of these studies, the DNA-damage is considered to be due to methacrylates binding to GSH resulting in increased ROS and subsequent DNA damage [36,38]. However, several other mechanisms have also been proposed to be involved in toxicity to methacrylates, such as binding to proteins through their cysteine residues or with other molecules containing nucleophilic groups, and direct binding to DNA [39].

This study shows that methacrylates have different potential to induce toxicity and that the ability to deplete GSH does not appear to be the main factor. We cannot rule out a genotoxic potential of MMA, although, it could indicate that if DNA damage is induced, the capacity of repair mechanisms was not exceeded.

CONCLUSION

Our results indicate that MMA can bind to GSH, probably to the cysteine residue, thereby weakening the oxidative defense in exposed organisms. The increase in ROS caused by the sequestering of GSH seems only partly to be responsible for the observed toxicity of MMA.

The possibility of complex formation between MMA and macromolecules should be investigated further.

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