THE EFFECTS OF METHYL METHACRYLATE MONOMER ON TESTOSTERONE LEVEL IN MALE RATS. An experimental study
http://www.lebanesemedicaljournal.org/articles/56-1/original2.pdf

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RÉSUMÉ • INTRODUCTION : Le méthacrylate de méthyle (MMA) est très souvent utilisé en médecine et en dentisterie. Ses effets indésirables sont bien décrits dans la littérature. Des lésions sur de nombreux organes sont associées à son utilisation. De nombreuses études expérimentales sur animal en confirment son effet toxique. Cependant son implication sur la fertilité masculine n’a pas été décrite à ce jour.


INTRODUCTION

Methyl methacrylate (MMA), a monomer of acrylic resin, has a wide variety of dental, medical and industrial applications [1-2]. It has been known for its use in denture bases, as well as in many kinds of dental, medical and industrial products. MMA, methacrylate acid and other
methacrylates readily polymerize to form long-chain homopolymers and co-polymers. MMA monomer is the most important ester of methacrylic acid commercially in use [3-4]. Special methacrylate polymers are used for dental prostheses; despite its widespread use in dentistry, MMA has been reported to be released into bloodstream during the surgery of total hip replacement and to cause embolism [4-6].

MMA monomer also appears to be toxic in the dental workplace. Several body systems appear to be affected including the skin, the respiratory tract, and the neurological system [6-11]. Dentists and other dental staff who work with this material may be exposed 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.

Other authors have investigated the effects of exposure to MMA on pregnancy mice during the period of gestation and have found an inhibition of body weight gain and significantly lower fetal, placental and maternal organs weights.

However, so far there is no study indicating the direct implication of the MMA on fertility mechanism.

METHODS

Sixty Sprague-Dawley rats weighing between 220 and 400 g were used. All rats were examined and were free of any disease. The rats were randomly divided into five groups, Group I (n = 15) consisted of control animals, Group II (n = 15) was exposed to MMA mixed with water at a concentration of 4‰ (v/v), Group III (n = 10) was exposed to MMA mixed with water at a concentration of 8‰ (v/v), Group IV (n = 10) was exposed at a concentration of 16‰ (v/v) and Group V (n = 10) at 32‰ (v/v).

The animals were examined for signs of any disease prior to exposure to MMA and were housed in colony cages (five per cage), so 12 cages were obtained (Group I : 3 cages, Group II : 3 cages, Group III : 2 cages, Group IV : 2 cages, Group V : 2 cages). In each cage, in order to identify the rats, perforations were made in the ears of the 5 rats, one perforation on right ear (6RE), one perforation in left ear (6LE), two perforations in right ear (6RE), two perforations in left ear (6LE) and one perforation in right and left ear (6RE 6LE). Temperature (between 20°C to 23°C), humidity (between 50% to 70%) in the animal room were automatically controlled as well as daylight timing of 9 hours which are a must for optimal health conditions and survival of the rats.

The daily food intake was 25 grams per rat consisting of 18% protein, 3% fat, 5.5% cellulose and 7.5% ash (Crispy rat : Verselle-Laga Belgium). Blood samples were collected from the tail before the exposure. At 8 months, which was the exposure duration, each animal was sacrificed and blood was obtained from the aorta. The usual precautions for venipuncture were taken. Whole blood was centrifuged at 3000 ppm for 10 minutes, after serum was separated.

The sera collected before the experimentation (n = 60) and those collected at the end of the study (n = 55) were stored at 2-8°C for 24 hours before performing the EIA. Five rats died, 2 from the control group and 3 from the experimental groups. All the samples were thawed and mixed thoroughly by gentle swirling prior to use.

The DSL-10-4000 active testosterone enzyme immunoassay (EIA) kit (Numelab) was used and the procedure followed the basic principle of enzyme immunoassay where there was competition between an unlabeled antigen and an enzyme-labeled antigen bound to the antibody binding sites. The amount of enzyme-labeled antigen bound to the antibody is inversely proportional to the concentration of the unlabeled analyst present. Unbound materials were removed by decanting and washing the wells. The absorbance measured was inversely proportional to the concentration of testosterone present in the serum. A set of testosterone standards is used to plot a standard curve of absorbance versus testosterone concentration from which the testosterone concentrations in the unknowns can be calculated.

Assay procedure

All the samples and reagent were allowed to reach room temperature and were mixed thoroughly by gentle immersion before use. Standards, controls and unknowns were assayed in duplicate. The micro titration strips were marked, 50 μl of the standards, controls and unknowns were pipetted into appropriate wells, the enzyme conjugate solution was diluted in the conjugate diluents. 100 μl of the enzyme conjugate solution and 100 μl of the testosterone antiserum were added to each well using a semi-automatic dispenser. The wells were shaken at a fast speed (500-700 rpm) on an orbital micro plate shaker at -25°C for one hour. Each well was aspirated and washed five times with the wash solution using automatic micro plate washer. 100 ml of the TMB chromogen solution was added to each well using a semi-automatic dispenser. The wells were shaken again at a fast speed (500-700 rpm) for 30 minutes at -25°C, 100 ml of the stopping solution (0.2 M sulfuric acid) was added to each well, then the absorbance of the solution was readied in the well within 30 minutes using a micro plate reader set to 45 minutes. After that, log-linear graph paper was used and the testosterone concentrations were determined.
from the standard curve, any samples readied higher than the highest standard was appropriately diluted with the 0 ng/ml standards and reassayed, and any sample readied lower than the lowest standard was reported as such.

**Statistical analysis**

Statistical significance between the two dates was assessed in each group by paired samples statistical test with the SPSS software. A $p$-value less than 0.05 was defined as statistically significant.

**RESULTS**

The daily administration of MMA for 8 months did not significantly change the rats' food consumption. The average food intake either in control or MMA treated rats was approximately 20 g/24 hr/rat. MMA administration did not influence the weight of animals. At the start of experimentation the body weight amounted to 258 ± 16 g and 260 ± 20 g in control and MMA treated rats, respectively. At the end of the experiment the corresponding values were 365 ± 20 g versus 375 ± 20 g in normal and MMA treated rats respectively. Similarly MMA administration did not change the rats’ water consumption.

The testosterone levels of the control Group I showed that the average value decreased from 2.79 ± 0.64 ng/ml to 1.54 ± 0.84 ng/ml without exposure to MMA. The two dates values were compared using paired samples test; $p = 0.001$ (< 0.05) was considered statistically significant.

The levels of Group II showed that the average value also decreased from 1.86 ± 0.51 ng/ml to 0.60 ± 0.36 ng/ml eight months after exposure to MMA. $p = 0.000$ (< 0.05) was considered statistically significant.

The levels of Group III showed no significant differences in the values before and after the experimentation, from 3.24 ± 0.43 ng/ml to 3.10 ± 0.62 ng/ml. $p = 0.35$ (> 0.05) was considered statistically non significant.

Contrary to the other results, the levels of Group IV showed an important increase of the values from 1.31 ± 0.52 ng/ml to 2.10 ± 0.72 ng/ml 8 months after exposure to MMA. $p = 0.003$ (< 0.05) was considered statistically significant.

The levels of Group V showed that the average value decreased again from 1.38 ± 0.6 ng/ml to 0.39 ± 0.44 ng/ml. $p = 0.002$ (< 0.05) was considered statistically significant.

Table I lists the summary of the variation values of all the groups and highlights that the testosterone level varies according to the concentration of the MMA mixed with water.

**DISCUSSION**

The chronic toxicity of MMA has been examined by various routes of exposure in rodents. MMA was found to be responsible for several changes in blood parameters [7].

After oral administration of MMA to animals at low concentration (8 nmole/kg) [8], the maximum accumulation of MMA in the serum was observed between 10 and 15 min after its administration. Then the MMA concentration declined steadily reaching a very low level after one hour. Few hours later, MMA could not be detected in rat serum. These results suggest that MMA is very quickly absorbed from the intestine, and rapidly degraded to methacrylate acid and methanol and very efficiently removed from the serum by a nonspecific enzyme carboxyl-esterase [8].

No histopathologic changes were found in liver as well as in serum activity of enzymes and hormones intoxicated with MMA. The liver is one of the organs in which methacrylate is metabolized [9].

This study showed that the testosterone blood level decreased by 44.8% in 8 months (from 2.79 ng/ml to 1.54 ng/ml) in mean value in male rats, which could be attributed to the normal ageing process.

If not exposed to MMA the four experimental groups would have displayed in 8 months a decrease of 44.8% in their testosterone level like the control group.

Concerning Group II, the exposure to MMA at 4‰ decreased the testosterone level by 67.7% i.e. 43.4% less than expected (0.60 ng/ml instead of 1.06 ng/ml) in case of non exposure to MMA. For Group III, the exposure of MMA at 8‰ decreased the testosterone level by 4.32% i.e. 73.1% more than expected (3.10 ng/ml instead of 2.08 ng/ml).

### Table I

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Before exposure (ng/ml)</th>
<th>After exposure (ng/ml)</th>
<th>Variation (%)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2.79 ± 0.64</td>
<td>1.54 ± 0.3</td>
<td>↓ 44.8 %</td>
<td>0.001</td>
</tr>
<tr>
<td>II</td>
<td>1.86 ± 0.51</td>
<td>0.60 ± 0.41</td>
<td>↓ 67.7 %</td>
<td>0.000</td>
</tr>
<tr>
<td>III</td>
<td>3.24 ± 0.43</td>
<td>3.10 ± 0.29</td>
<td>↓ 4.32 %</td>
<td>0.35</td>
</tr>
<tr>
<td>IV</td>
<td>1.31 ± 0.52</td>
<td>2.10 ± 0.28</td>
<td>↑ 60 %</td>
<td>0.003</td>
</tr>
<tr>
<td>V</td>
<td>1.38 ± 0.6</td>
<td>0.39 ± 0.17</td>
<td>↓ 71.7 %</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Values are means ± SEM & $p < 0.05$ is statistically significant. ↓ decrease ↑ increase*
Effects of MMA on testosterone level in male rats

Concerning Group IV, the exposure to MMA at 16‰ increased the testosterone level by 60% i.e. 187.6% more than expected (2.10 ng/ml instead of 0.73 ng/ml) in case of non exposure to MMA. While for Group V, the exposure of MMA at 32‰ decreased the testosterone level by 71.7% i.e. 49% less than expected (0.39 ng/ml instead of 0.77 ng/ml) in case of non exposure to MMA (Table II, Figure 1).

The study shows that the exposure of rats to low (4‰) and high (32‰) concentrations accelerates the decrease of testosterone level in the blood while the concentration of 8‰ kept the testosterone level constant (3.10 ng/ml) for the whole 8 months , whereas we expected it to go down (1.79 ng/ml). At 16‰, amazingly, the testosterone level increased by 187.6% from the expected value (0.73 ng/ml).

These results corresponded with another experimental study which examined the effects of exposure to MMA vapor on pregnancy. Mice proved to be pregnant were exposed to 0, 2, 20 and 100 ppm MMA continuously for 24 hours during the period from day 0 to day 15 of gestation. While the 20 ppm group showed significantly lower ovary and placental weights, 0, 2 and 100 ppm groups showed no abnormality (Nicholas, 1979) [10]. In addition to these results, in vivo as well as in vitro experimental studies, there have been a number of epidemiological studies, which have examined the possible genotoxicity and mutagenicity of MMA exposure [11-15]. A study of 38 male workers who were exposed to MMA at concentrations between 0.9 ppm to 71.0 ppm examined chromosome aberration rates. The results showed that occupational MMA exposure was not associated with genotoxicity ; this occurred only under the conditions studied (Kim, 1994 ) [16].

From a practical point of view it was also interesting to check methacrylate uptake by damaged liver. The histological features of D-galactosamine liver suggest a striking similarity to the histopathological changes in acute human viral hepatitis. Thus, the kinetics of MMA elimination were also investigated in the liver obtained from rats treated with galactosamines. Pretreatment of rats with galactosamines caused a significant decrease of MMA uptake by perfused liver. This suggests that only intact liver is able to accumulate methacrylate efficiently [17].

Leggate et al. (2003) suggested that the inhibition of serum carboxylesterase by the MMA may be also an important factor of enhancement of MMA toxicity since hydrolytic degradation can be diminished [17].

**TABLE II**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Before exposure (ng/ml)</th>
<th>Expected values if no exposure 8 months later (ng/ml)</th>
<th>After exposure 8 months later (ng/ml)</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control 2.79 ± 0.64</td>
<td>↓ 44.8 % 1.54 ± 0.3</td>
<td>―</td>
<td>―</td>
</tr>
<tr>
<td>II</td>
<td>4‰ MMA 1.86 ± 0.51</td>
<td>↓ 44.8 % 1.06 ± 0.21</td>
<td>↓ 67.7 % 0.60 ± 0.41</td>
<td>↓ 43.4 %</td>
</tr>
<tr>
<td>III</td>
<td>8‰ MMA 3.24 ± 0.43</td>
<td>↓ 44.8 % 1.79 ± 0.11</td>
<td>↓ 4.32 % 3.10 ± 0.29</td>
<td>↑ 73.1 %</td>
</tr>
<tr>
<td>IV</td>
<td>16‰ MMA 1.31 ± 0.52</td>
<td>↓ 44.8 % 0.73 ± 0.22</td>
<td>↑ 60 % 2.10 ± 0.28</td>
<td>↑ 187.6 %</td>
</tr>
<tr>
<td>V</td>
<td>32‰ MMA 1.38 ± 0.6</td>
<td>↓ 44.8 % 0.77 ± 0.17</td>
<td>↓ 71.7 % 0.39 ± 0.17</td>
<td>↓ 49 %</td>
</tr>
</tbody>
</table>

*Values are means ± SEM ↓ decrease ↑ : increase

1.79 ng/ml in case of non exposure to MMA.

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**CONCLUSION**

This preliminary research presumed possible that MMA mixed with water at the concentrations we studied acts on specific liver cells and that the methacrylate elimination from blood could be substantially diminished. Consequently, the effector functions of the testosterone secretion could be affected.

However, the mechanism whether by the MMA in blood interferes with testosterone secretion should be determined in the future using histological studies to try to explain the modification of testosterone level in correlation with MMA concentration mixed with water.
ACKNOWLEDGEMENTS

The authors would like to thanks Professor Patrick Fenichel (France) for providing information and advice and Doctor Mario Abdallah for the statistical analysis.

REFERENCES


