

THE EFFECTS OF METHYL METHACRYLATE MONOMER ON TESTOSTERONE LEVEL IN MALE RATS. An experimental study

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Fakhouri J, Aftimos G, Hilal G, Sarkis R. The effects of methyl methacrylate monomer on testosterone level in male rats. An experimental study. *J Med Liban* 2008 ; 56 (1) : 11-15.

ABSTRACT • INTRODUCTION : Methyl methacrylate (MMA) is commonly used in medicine and dentistry. The adverse effects of MMA are well described in the literature. Animal studies have largely confirmed the risks reported in clinical observations. There is no study indicating direct implication of MMA on male fertility mechanism.

OBJECTIVES : The purpose of this study was to determine whether MMA is able to modify the testosterone level.

METHODS : The target population consisted of 60 male Sprague-Dawley rats. They were closed in colony cages and divided into five groups : The first group (n = 15) designated as the control group and four experimental groups (n = 45).

Experiments were conducted by exposing the four experimental groups to MMA with water at different concentrations (4‰, 8‰, 16‰ and 32‰) administered *per os*. The exposure duration was eight months. Blood was obtained before and at the end of the exposure and the measurement of the testosterone level was made by EIA test.

RESULTS : The exposure of rats at a moderate concentration of MMA (16‰) showed an increase in testosterone level of 60% ($p = 0.003$) while the other groups showed a decrease of testosterone level. The control group showed a decrease of 44.8% ($p = 0.001$), the rats exposed at 4‰ showed a decrease of 67.7% ($p = 0.000$), those exposed at 8‰ showed a decrease of 4.32% ($p = 0.35$), the rats exposed at 32‰ showed a decrease of 71.7% ($p = 0.002$).

CONCLUSION : Despite the fact that MMA at low concentration was rapidly hydrolyzed in blood due to the nonspecific carboxylesterase and metabolized at high concentration by the liver, its effects on testosterone level were significant. These preliminary results showed an interference of the MMA with the testosterone hormonal equilibrium that could be an interesting target for further investigations.

Fakhouri J, Aftimos G, Hilal G, Sarkis R. Les effets du monomère méthacrylate de méthyle sur le taux de testotérone chez le rat. Etude expérimentale. *J Med Liban* 2008 ; 56 (1) : 11-15.

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 confirmant son effet toxique. Cependant son implication sur la fertilité masculine n'a pas été décrite à ce jour.

OBJECTIF : Etudier l'effet du MMA, administré mélangé à l'eau, sur le taux de testostérone dans le sang.

MÉTHODES : Soixante rats mâles Sprague-Dawley ont été utilisés et divisés en 5 groupes. Le 1^{er} groupe (n = 15) étant le groupe témoin et 4 groupes (n = 45), ceux de l'expérimentation. Les rats ont été hébergés dans un centre spécialisé suivant les recommandations internationales d'animalerie. De l'eau mélangée à du MMA a été administrée à 4 différentes concentrations (4‰, 8‰, 16‰ et 32‰). La durée de l'exposition a été de 8 mois. Du sang de chaque rat a été prélevé de la queue avant l'expérimentation et 8 mois plus tard de l'aorte. Le taux de testostérone a été mesuré par le test EIA.

RÉSULTATS : Seule l'administration du MMA à une concentration modérée (16‰) entraîne une augmentation du taux de testostérone dans le sang de 60% ($p = 0,003$) alors que tous les autres groupes ont montré une baisse plus ou moins importante du taux de testostérone. Le groupe contrôle a montré une baisse du taux de 44,8% ($p = 0,001$), les rats exposés à 4‰ une baisse de 67,7% ($p = 0,000$), ceux exposés à 8‰ une baisse de 4,32% ($p = 0,35$), alors que ceux exposés à 32‰ ont montré une baisse de 71,7% ($p = 0,002$).

CONCLUSION : Bien que le MMA à faible concentration soit métabolisé par l'enzyme non spécifique carboxylestérase et par le foie, essentiellement à forte concentration, son effet sur le taux de testostérone est notable. La toxicité du MMA pourrait être en rapport avec des lésions du foie dues au produit lui-même. Ces résultats préliminaires montrent que l'administration du MMA interfère avec l'équilibre hormonal de la testostérone ce qui constitue une cible intéressante à explorer.

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

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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 abnormalities or lesions in several organs. MMA released from acrylic resin dentures into the digestive tract has often been postulated as a cause of denture sore mouth.

In the years since its introduction, there have been a large number of reports concerning the complications and untoward side-effects associated with its use ; these range from a reported bladder fistula, contact dermatitis, allergic stomatitis, to serious life-threatening episodes of respiratory dysfunction and cardiovascular pathologies. These cardiovascular effects range from marked hypotension and cardiac arrhythmias to a reported coronary 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 (₁RE), one perforation in left ear (₁LE), two perforations in right ear (₂RE), two perforations in left ear (₂LE) and one perforation in right and left ear (₁RE ₁LE). 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 serums 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 antigen 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 pipeted 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 µl 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 µl 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 carboxylesterase [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

TABLE I
TESTOSTERONE LEVEL (ng/ml) BEFORE AND AFTER EXPOSURE TO METHYL METHACRYLATE (MMA) IN ALL MALE RATS GROUPS*

GROUP		Before exposure (ng/ml)	After exposure (ng/ml)	Variation (%)	<i>p</i> -value
I	Control	2.79 ± 0.64	1.54 ± 0.3	↓ 44.8 %	0.001
II	4‰ MMA	1.86 ± 0.51	0.60 ± 0.41	↓ 67.7 %	0.000
III	8‰ MMA	3.24 ± 0.43	3.10 ± 0.29	↓ 4.32 %	0.35
IV	16‰ MMA	1.31 ± 0.52	2.10 ± 0.28	↑ 60 %	0.003
V	32‰ MMA	1.38 ± 0.6	0.39 ± 0.17	↓ 71.7 %	0.002

*Values are means ± SEM & *p* < 0.05 is statistically significant. ↓ decrease ↑ increase

TABLE II
MEAN VARIATION IN TESTOSTERONE LEVEL IN MALE RATS EXPOSED OR NOT TO MMA*

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

*Values are means ± SEM ↓ decrease ↑ : increase

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

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].

Leggat 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].

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.

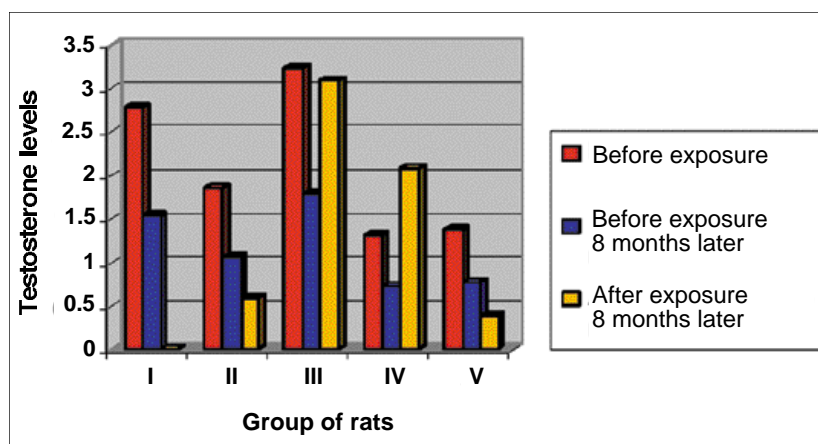


FIGURE 1

Histogram : A comparison between the original testosterone level, the usual result after 8 months in the case of no exposure to MMA and after 8 months of exposure.

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تأثيرات الميثيل ميثاكريلات احادية التقسيم MMA على مقدار التستسترون. دراسة مخبرية على ذكور الجرذان.

موجز : MMA كثيرا ما يستعمل في الجراحة العصبية وجراحة العظم وطب الاسقان وصفت تأثيرات غير مرغوب فيها بالمنشورات الطبية اذ اظهرت عدة افات على اعضاء بسبب استعمالها واكدت الدراسات التجريبية على الحيوان من عدة مستعملين سمية هذا العقار.

الموضوع - دراسة MMA المعطى ممزوجا مع الماء اظهرت تأثيره على التستسترون في الدم، الطرق - 60 جرذا ذكرا Sprague - Dawley استعمل خمسة عشر منها في قفص خاص وهي المجموعة الاولى للمقارنة واربع مجموعات (عدد 45) اجريت عليها التجربة. وضع الجرذان في مكان خاص استنادا الى توصيات اختصاصيي الحيوان. مزيج الماء مع MMA اعطي باربع تركيبات (4-8-16-32 بالالف) وكانت مدة التعرض ثمانية شهور، وقبل التجربة اخذ الدم من كل جرذ من ذيله وبعد ثمانية شهور اخذ الدم من الابهر جرى فحص مقدار التستسترون باختبار EIA. النتائج - اعطاء MMA بتركيز معتدل 16 بالالف ادى الى زيادة التستسترون في الدم بنسبة 60٪ (احتمال 0,003) بينما بقية المجموعات اظهرت نقصا اكثر او اقل اهمية للتستسترون. اظهرت مجموعة المقارنة نقصا بمقدار 44,8٪ (احتمال 0,001) اما المجموعة التي اعطيت 4 بالالف فقد ابدت نقصا بمقدار 67,7٪ (احتمال 0,000) والمجموعة التي اعطيت 8 بالالف ابدت نقصا بمقدار 4,32٪ (احتمال 0,35) اما مجموعة 32 بالالف فكان النقص عندها 71,7٪ (احتمال 0,002) الخلاصة - على الرغم من ان MMA تم استقلابه بتركيزات ضعيفة بانزيم غير نوعي كاربوكسيلستراز، وبالكبد خاصة بتركيزات عالية فان تأثيره على التستسترون كثير ي اهمية. وقد تكون سمية MMA سببا لأفات كبدية. اظهرت هذه النتائج الاولى ان اعطاء MMA يتداخل مع التوازن الهرموني للتستسترون فيكون هدفا هاما لبحته.