

Assessment of methyl methacrylate vapor toxicity on the rat tracheal epithelium

Análise da toxicidade dos vapores de metil metacrilato sobre o epitélio traqueal de ratos

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ABSTRACT: Methyl methacrylate (MMA) is a monomer that is polymerized into resin by light and heat, producing a clear, resistant, durable and relatively inert plastic material. Because of these characteristics, MMA is largely used in Medicine as bone cement and in Dentistry, in dental braces and prostheses, thus generating continuous interest in its toxicity. Experimental and clinical studies have documented that monomers may cause a wide range of adverse health effects. The most important occupational exposure route of MMA is by inhalation. This study aims to evaluate the toxicity of MMA to the tracheal epithelium, according to the time of exposure. For this purpose, two experimental groups of rats were exposed to MMA by inhalation under poor ventilation: one group (n = 36) was exposed permanently, and the other (n = 36) was exposed during 8 hours per day, without water and food supply during the exposure period. A control group (n = 8) received normal air supply. Twelve animals of each study group were sacrificed after 5, 8 and 10 days of exposure together with two or four control animals. Twenty-nine (80.5%) of the rats continuously exposed to MMA developed inflammation on the tracheal epithelium, as well as 58.33% (n = 21) of those exposed 8 h/day and 87.5% (n = 7) of the control rats. No association was observed between the inflammatory process and MMA exposure; no significant alterations in the tracheal epithelium thickness were observed. Further studies on longer exposure times and analysis of other parameters will have to be conducted to exclude the possibility of tracheal damage by vapors of MMA.

DESCRIPTORS: Methylmethacrylate; Trachea; Toxicity.

RESUMO: O metil metacrilato (MMA) é um monômero que se polimeriza em resina pela ação da luz e do calor, transformando-se em plástico claro, resistente e durável, relativamente inerte. Por apresentar tais características, o MMA tem sido muito usado na Medicina, como cimento ósseo, e na Odontologia, em aparelhos e próteses dentais, o que tem suscitado interesse na avaliação de sua toxicidade. Estudos experimentais e clínicos têm mostrado que os monômeros podem causar uma gama de efeitos adversos. A principal via de exposição ocupacional ao MMA é a inalatória. Este trabalho visa a avaliar a ação tóxica do MMA sobre o epitélio traqueal em relação ao tempo de exposição. Para isso, dois grupos experimentais de ratos foram expostos ao MMA por inalação, com restrição de ventilação: um grupo (n = 36) foi exposto continuamente, e outro (n = 36) foi exposto durante oito horas diárias, sem água e comida durante o período de exposição. Um grupo controle (n = 8) recebeu ar normal. Doze animais de cada grupo de estudo foram sacrificados com 5, 8 e 10 dias de exposição, junto com dois ou quatro animais do grupo controle. Vinte e nove (80,5%) dos ratos expostos continuamente ao MMA apresentaram inflamação do epitélio traqueal, assim como 58,33% (n = 21) daqueles expostos 8 horas/dia e 87,5% (n = 7) dos controles. Não se observou associação entre o processo inflamatório e a exposição ao MMA, nem alterações significativas na medida da espessura do epitélio traqueal. Novos estudos, com tempo mais prolongado de exposição e análise de outros parâmetros, devem ser realizados para que seja excluída, totalmente, a possibilidade de dano traqueal por vapores de MMA.

DESCRIPTORES: Metilmetacrilato; Traquéia; Toxicidade.

INTRODUCTION

Methyl methacrylate (MMA) has been used over the last 50 years for acrylic resin production

and has a wide range of application in plastics and the building industry, as well as in Medicine and Dentistry¹¹.

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Acrylic polymers were introduced into Dentistry in 1937, and by 1946 it was estimated that 98% of the dental materials were made of MMA⁹.

Deichmann⁴ (1941) was the first to have a study about the environmental damages caused by MMA published and to test some of its administration routes (oral, subcutaneous, inhalational and cutaneous).

In Medicine and Dentistry, the main professional exposure route is inhalation¹; the Occupational Safety and Health Code established the average of 8 hours permissible to MMA exposure at 100 ppm²; exposure at higher levels can generally cause injuries from local ocular and mucosal irritation, including stomatitis, to asthma and systemic effects such as neurologic disturbances^{5,8,12,16,22}.

The toxicity of MMA to patients has been largely studied. However, not many studies have been conducted to demonstrate its potential hazardous property to persons exposed to its vapor, especially technicians who work in prosthesis production facilities^{13,14}.

As MMA is largely used in dental prosthesis production and due to its great volatility, this study was delineated to investigate its potential toxic action on the tracheal epithelium and its relationship to the time of exposure.

MATERIAL AND METHODS

We selected 80 Wistar albino rats aged 90 days, 40 males and 40 females, which were grouped in a number of three per cage and put in different shelves in a place controlled for temperature and humidity and equipped with 12-hour dark-light cycle.

The animals were divided into three groups: one group continuously exposed to MMA (n = 36), a group exposed to MMA for 8 hours per day without water and food intake (n = 36), and a control group (n = 8), which was kept in a separate place.

The plastic boxes in which rats were placed had an upper grate covered by a piece of white non recycled plastic over three quarters of its surface (ventilation restricted). Amber flasks (Nadir Figueiredo Indústria e Comércio, São Paulo, Brazil) of 100 ml capacity and having a 2-cm-diameter opening with perforated cover were supplied with 10 ml of MMA (99.9% obtained from CLÁSSICO - Brazilian Industry Limited, São Paulo, Brazil) and placed in each box, fixed to the grate.

After 5 and 8 days of exposure, 12 rats (6 males and 6 females) of each group were sacrificed together with two control animals. After 10 days of

exposure, 12 rats of each group and four controls were sacrificed.

Sacrifice was carried out with 2 ml of thiopental (Abbott Laboratories, Chicago, USA) administered in the peritoneal cavity and necropsy was performed. An annular fragment from the medium third of the trachea was extracted.

The material collected was routinely processed, fixed in 10% formalin (Cinética Indústria Química, São Paulo, Brazil) during 24 hours, embedded in paraffin (Dinâmica Reagentes Analíticos, São Paulo, Brazil), sectioned (in sections of 5 µm), and stained with Hematoxylin and Eosin (H&E) (Dolles, São Paulo, Brazil).

Measurements of the tracheal epithelium thickness were carried out in four areas of each fragment using an image analysis system (IMAGELAB 2000, supplied by the University of São Paulo, Brazil) (Figure 1).

Statistical analysis was conducted by means of analysis of variance, and two factors (exposure procedure and sacrifice period) were considered. Gender was related to the thickness of the tracheal epithelium. For the categoric variable, tracheal inflammation, the chi-squared test was performed for the independent samples in order to check its association with exposure procedure, sacrifice period and gender.

RESULTS

Histopathological analysis of the trachea

An inflammatory process in the submucosa predominantly composed of lymphocytes with rare plasma cells and neutrophils was observed (Figure 2).

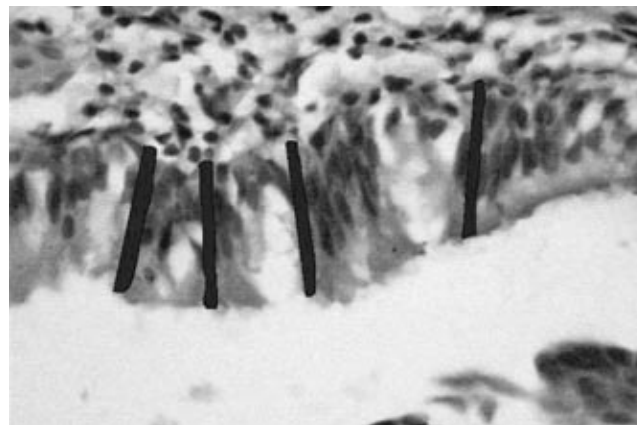


FIGURE 1 - Measurement pattern of tracheal epithelium thickness – control female rat (IMAGELAB - Hematoxylin - Eosin, 40 X).

Among the rats continuously exposed to MMA, 80.5% (n = 29) had tracheal epithelium inflammation. So did 58.33% (n = 21) of the 8 h/day exposure group, and 87.5% (n = 7) of the control animals (Table 1). An inflammatory process was observed in seventeen (65.38%) of them after 5 days of exposure (Table 2).

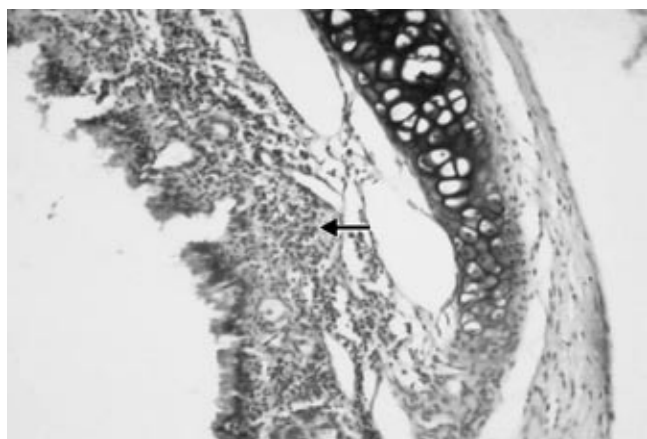
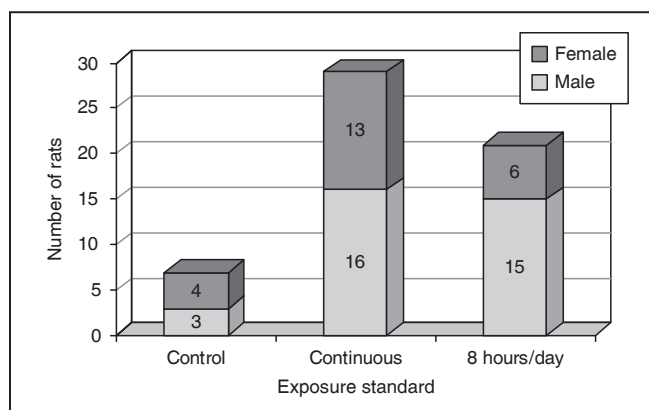


FIGURE 2 - Photomicrograph of trachea showing chronic inflammation of the submucosa (arrow) - 5 days of continuous exposure, male rat (Hematoxylin - Eosin, 10 X).

TABLE 2 - Relation (number of rats) between tracheal inflammation and sacrifice period.

| Sacrifice period | Tracheal inflammation | | |
|------------------|-----------------------|--------|-------|
| | Present | Absent | Total |
| 5 days | 17 | 9 | 26 |
| 8 days | 20 | 6 | 26 |
| 10 days | 20 | 8 | 28 |
| Total | 57 | 23 | 80 |



GRAPH 1 - Distribution of rats with tracheal inflammation according to exposure type and gender (n = 57).

Thirty-four (85%) of the male rats and only 23 (57.5%) of the female rats had tracheal inflammation (Table 3 - Graphs 1 and 2). A significant association between sex and presence of tracheal inflammation ($p = 0.007$) was observed, but not between gender and type of exposure ($p = 0.064$) and between gender and sacrifice period ($p = 0.655$).

Thickness measurement of tracheal epithelium

Due to technical difficulties, 3 cases were excluded from this analysis: 2 female controls and one female rat exposed to MMA for 8 hours/day.

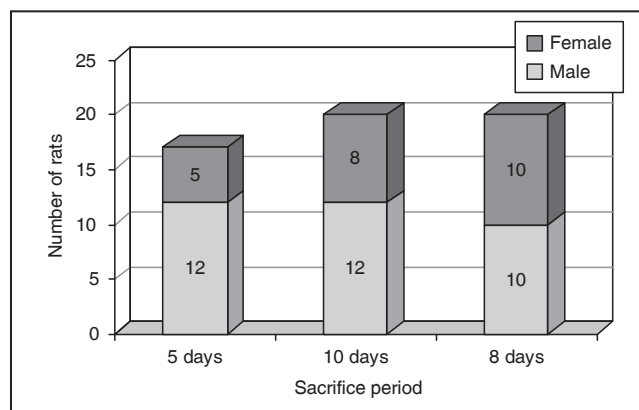
There were no significant differences for this parameter among the different groups ($p > 0.05$) (Graph 3).

TABLE 1 - Relation (number of rats) between tracheal inflammation and exposure type.

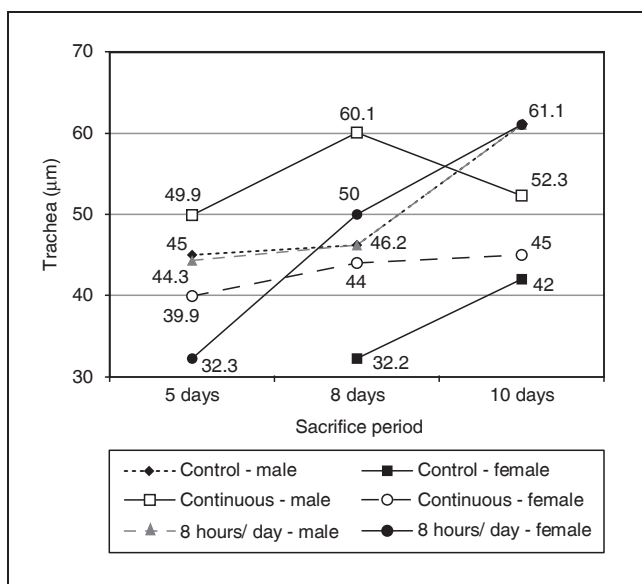
| Exposure type | Tracheal inflammation | | |
|---------------|-----------------------|--------|-------|
| | Present | Absent | Total |
| Control | 7 | 1 | 8 |
| Continuous | 29 | 7 | 36 |
| 8 hours/day | 21 | 15 | 36 |
| Total | 57 | 23 | 80 |

TABLE 3 - Relation (number of rats) between tracheal inflammation and gender of animals.

| Gender | Tracheal inflammation | | |
|--------|-----------------------|--------|-------|
| | Present | Absent | Total |
| Male | 34 | 6 | 40 |
| Female | 23 | 17 | 40 |
| Total | 57 | 23 | 80 |



GRAPH 2 - Distribution of rats with tracheal inflammation according to sacrifice period and gender (n = 57).



GRAPH 3 - Variation of the tracheal epithelium thickness in rats related to exposure type and sacrifice period and classified per gender (n = 77).

DISCUSSION

MMA derives from methacrylic acid. It has the structural formula observed in Figure 3 and a molecular weight of 100, specific gravity of 0.936, boiling point of 100 - 101°C and refraction index of 1.413 at 20°C⁴.

It is transformed into resin by light, heat, oxygen and oxygenated compounds, and has great applicability at this polymerized state^{1,9}. Acrylic products resulting from this polymerization and in accordance with recommended technical rules do not cause tissue damage and are of low toxicity to patients⁹.

Due to its volatility, occupational exposure occurs by inhalational route, being first hydrolyzed in the nasal cavity by the carboxylesterase enzyme^{13,18}.

The exposure of prosthetic laboratory workers to these monomers has been studied in the last decades, but few studies have been conducted to evaluate the potential damage of MMA on several tissues. It has been reported that MMA is not carcinogenic^{11,18} or teratogenic¹⁹.

Many studies that evaluate damage to the respiratory tract, specially to the nasal cavity, report inflammation, glandular atrophy, focal hyperplasia of basal cells and squamous metaplasia of the nasal cavity epithelium^{1,11,13}.

MMA effects on olfactory tissue show a pattern of regeneration during continuous exposure

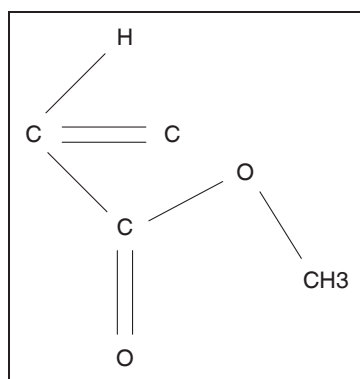


FIGURE 3 - Structural formula of methyl methacrylate.

and degeneration that originates disorganization and an apparent resolution⁷, also noticeable in exposure to other chemical agents^{6,10}.

Only Tansy *et al.*^{20,21} (1980, 1979) studied injuries to the tracheal epithelium. Using light microscopy, they noticed only isolated hemorrhagic foci, but when submitting these areas to electron microscopy evaluation, they detected ciliary denudation and reduction in the number of microvilli.

In our study, we observed a predominantly lymphocytic mixed inflammatory infiltrate in the tracheal epithelium of exposed and control rats. This finding is not statistically significant concerning the analysis of inflammation and MMA exposure. The predominance of the inflammatory process in male rats, regardless of the exposure to MMA, is of statistical significance.

Tracheal epithelium thickness of MMA exposed rats proved similar to that of control rats, and cell adaptations such as atrophy and hypertrophy were not observed.

Although not experimentally demonstrated, it is believed that the toxicity of MMA to the respiratory tract mucosa is the result of a high activity of carboxylesterase enzymes and consequent formation of methacrylic acid, an irritant and chemical corrosive¹³.

Toxic injury associated with MMA vapors could at least be partially related to metabolites originated from carboxylesterase enzyme degradation, and not only to its direct action over tissues as demonstrated for ethyl acrylate toxicity¹⁵.

Carboxylesterase enzyme can be found in higher concentrations in the olfactory epithelium and in lower concentrations in the respiratory epithelium^{3,17}, which could possibly justify the absence of damage to the respiratory-type tracheal epithelium.

In 2001, Mainwaring *et al.*¹³ published a comparative study on the action of MMA in the nasal

epithelium of rats, hamsters and humans. They concluded that the latter were less susceptible to the toxic action of MMA than the former two.

Differences in nasal morphology between rats and humans and in the metabolism of MMA, such as a lower carboxylesterase enzyme activity in humans, suggest that humans might develop toxic lesions related to MMA exposure vapors in lower extent¹³. This has to be taken into account, since the findings of experimental studies with rats cannot be reliably considered for human beings.

The present study corroborates the findings of Tansy *et al.*^{20,21} (1980, 1979), who have shown that the damage to the tracheal epithelium could possibly be restricted to the cell microstructure and

lead to functional injuries such as ciliary motility alterations with a decrease in the elimination of mucus and, eventually, pulmonary and tracheo-bronchial infections.

CONCLUSION

Air concentration of MMA in dental prosthesis laboratories and operating room facilities where it is handled shall not exceed 100 ppm, and exhausting systems should be used following Occupational Safety and Health rules. Moreover, governmental policies should warrant supervision and control of industrial and health facilities that use MMA-derived resins in their manufacturing procedures.

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