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TISSUE REACTION TO METHYL METHACRYLATE MONOMER

A Comparative Study in the Rabbit's Ear on the Toxicity of Methyl Methacrylate Monomer of Varying Composition

LARS LINDER

Laboratory of Experimental Biology, Department of Anatomy, University of Göteborg, Sweden.

The aim of the present investigation was to evaluate if a bone cement monomer with a high concentration of accelerator (N, N-dimethyl-p-toluidine) is more toxic than a methyl methacrylate monomer, free from accelerator. 1) No difference in the acute local toxicity between CMW®, Simplex-P® and pure methyl methacrylate monomer was seen. 2) By gas chromatography, N, N-dimethyl-p-toluidine was shown to be water soluble to a small extent. Any bone cement monomer in current use can be fully dissolved in saline to a concentration of about 1 per cent.

Key words: bone cement monomer, local toxicity; N, N-dimethyl-p-toluidine, water solubility

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The monomer of today's acrylic bone cements is made up of methyl methacrylate (MMA) monomer. To this are added minute amounts of stabilizer (hydroquinone, ascorbic acid) and accelerator (N, N-dimethyl-p-toluidine). The cements most widely used are CMW®, Palacos® and Simplex®. Their monomers differ somewhat in composition (Table 1).

It is known that MMA monomer is cytotoxic (Hoppe 1956, Mohr 1958, Hulliger 1962, Schlag et al. 1973). It is also known that leakage of MMA monomer into the tissues takes place from the surface of the implanted bone cement (Homsy et al. 1972, Wenzl et al. 1973, Kutzner et al. 1974). The greater part of this monomer leakage takes place while the cement still has its pasty consistency, that is, during the very first minutes after implantation. After the cement is polymerized, only minute amounts of residual monomer are released for possible toxic action (Smith & Bains 1956, Lee et al. 1973). An acute local chemical tissue injury, thus, seems unavoidable, but its magnitude and its functional consequences are not fully known.

Hulliger (1962) has studied the effect in tissue cultures of Palacos® monomer and pure MMA monomer, and concluded that both have the same toxicity. However, more recent work on Palacos® has suggested that the accelerator, N, N-dimethyl-p-toluidine (NNDPT) makes Palacos® more toxic than pure MMA

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Table 1. Composition of the monomer of the most widely used types of bone cement as well as the pure monomer used in the study. The figures are provided by the respective manufacturers.

	N, N–dimethyl–p–toluidine	Hydroquinone	Ascorbic acid	MMA
Simplex-P ®	2.6 %	75 ± 15 PPM	—	balance
CMW ®	1.0–1.5 %	5 — 10 PPM	0.02 %	balance
Palacos ®	0.7 %	60 PPM	—	balance
Pure	–	0.1 PPM	—	balance

(Schlag et al. 1973). If such a difference in monomer toxicity is caused by the addition of NNDPT, it would be even more marked in the case of Simplex-P ® and CMW ®, since Simplex-P ® contains three to four times as much NNDPT as Palacos ®, and CMW ® twice as much (Table 1).

It is of practical importance to keep the monomer toxicity as low as possible. Therefore, it seems justified to evaluate if the Simplex-P ® and CMW ® monomers have a toxicity which obviously differs from that of pure MMA monomer. In the present study, these three monomers have been compared with regard to their local tissue effects. Palacos ® has an accelerator content intermediate to those of Simplex-P ® and pure MMA, and since its toxicity, therefore, can also be expected to be intermediate, Palacos ® has not been included in the study.

The microvascular system is a sensitive indicator of tissue injury, and microangiography of the rabbit's ear has proved valuable in studies on the local tissue irritancy of various drugs (Brånemark 1967, Brånemark et al. 1969). This technique gives a picture of the reparative process following tissue injury, but information on the acute tissue effect can also be gained.

MATERIAL AND METHODS

CMW ®, Simplex-P ® and pure MMA (kindly supplied by AB Bofors Nobelkrut, Sweden) monomer were used in the study. Four concentrations of each monomer were tested: 100 per cent monomer and 2 per cent, 1 per cent and 0.5 per cent v/v dilutions in isotonic saline (for technical details, see below).

Sixteen male, albino rabbits, weight 1.5 kg, age 3 months, were used. The animals were divided into four equal-sized groups, one group for each monomer concentration tested.

Each animal received two 0.05 ml injections subperichondrally on the outer surface of each ear, one injection proximally and one distally. Three of the four injections thus given to each animal contained the three monomers, the fourth (isotonic saline) serving as a control of the trauma produced by the injection procedure. In all groups, each monomer was injected proximally twice and distally twice.

In an additional series a 0.03 per cent v/v solution of N, N-dimethyl-p-toluidine (Fluka AG, Switzerland) in isotonic saline was compared to isotonic saline with regard to the local tissue reaction. Four rabbits were used. The NNDPT solution and the saline solution were alternately injected proximally and distally in the ears. Eight injections of each solution were made. The NNDPT concentration was chosen so that it would approximately equal the NNDPT concentration of the 1 per cent Simplex-P ® solution.

The animals were regularly inspected and the tissue reaction seen on transillumination of the ears recorded. After 8–10 days the animals were submitted to microangiography. Under urethane anaesthesia, 500 ml of a 50 per cent saline suspension of barium sulphate (Mixobar ®, Astra, Sweden) were infused into the abdominal aorta in the retrograde direction. The jugular veins were opened to balance the infused volume. Repeated injections of heparin and lidocain through the infusion catheter were made to facilitate the filling of small calibre vessels. The ears were then radiographed by means of a Machlett OEG-50 tube on Kodak MR plates at 15 mA, 12 kV and a film-focus distance of 12 cm. The exposure time was 15 minutes. Histological sections, 4 μ in thickness, stained with haemotoxylin-eosin, were taken from representative ears.

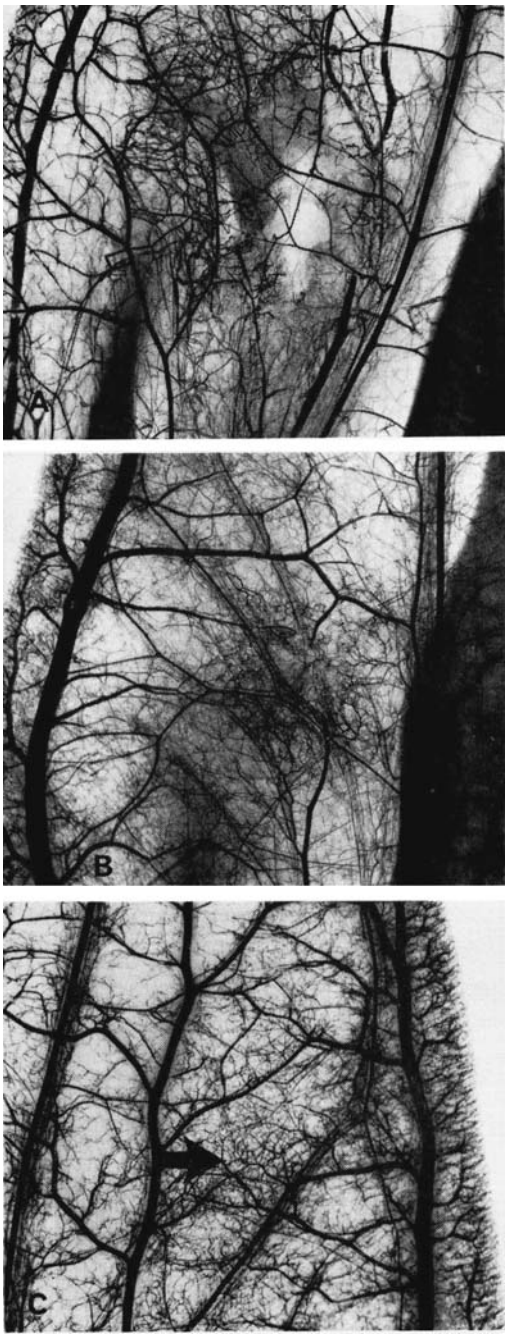

Figure 1. Microangiograms of rabbit ears exposed to different concentrations of MMA monomer. A. 100 per cent monomer. A large central area devoid of small vessels (indicating necrosis) with surrounding vascular reaction (typical of granulation tissue). B. 2 per cent monomer. Less extensive vascular reaction and more superficially located necrosis. C. 1 per cent monomer. A small area with a vascular architecture typical of granulation tissue is seen at the arrow.

Technical

The CMW® and Simplex-P® monomers were sterile from the manufacturer. The pure MMA monomer was not sterilized, and every attempt at membrane filtration failed as the filters were destroyed by the monomer. However, since the monomer is considered to be self-sterilizing (Charnley 1972), no further attempts were made. Once taken from their original containers, all three monomers were treated aseptically. Glass syringes were used throughout.

The 2, 1 and 0.5 per cent monomer concentrations were obtained by mixing 2, 1 and 0.5 ml of the 100 per cent monomer with 100 ml of isotonic saline. The 2 per cent mixture became opaque on shaking, whereas the others were clear.

The solubility in water of MMA monomer is 1.59 g/100 g at 20° C (Riddle 1954) and of hydroquinone 7.4 g/100 ml at 25° C (May & Baker Ltd, Dagenham, England). No figure on the solubility in water of NNDPT has been obtained.

In order to evaluate if the NNDPT of the monomers tested was dissolved or suspended in the saline, its solubility in a 1 per cent aqueous monomer solution was determined. (This part of the study was done by L. Harthon and L. Kullberg, AB Bofors Nobelkrut, Sweden).

A 1 per cent water solution of the pure MMA monomer was prepared. The solution was then saturated with NNDPT. Gas chromatography was performed with a Perkin Elmer F 11 gas chromatograph, equipped with a flame ionization detector. The operating conditions were as follows:

Column: ¼″ glass, packed with 20 per cent SE 30 on Chromosorb W.
Oven temperature: 130° C.
Injection temperature: 250° C.
Carrier gas: N_2.
Carrier gas flow rate: 40 ml/min.
Sample size: 1.0 µl.

The MMA peak was used as an internal standard and the NNDPT peak was related to that of the MMA in the subsequent calculations.

RESULTS

100 per cent monomer. On injection there was a marked tendency for the monomer to spread in the tissues. Within a minute, bleeding occurred in the area occupied

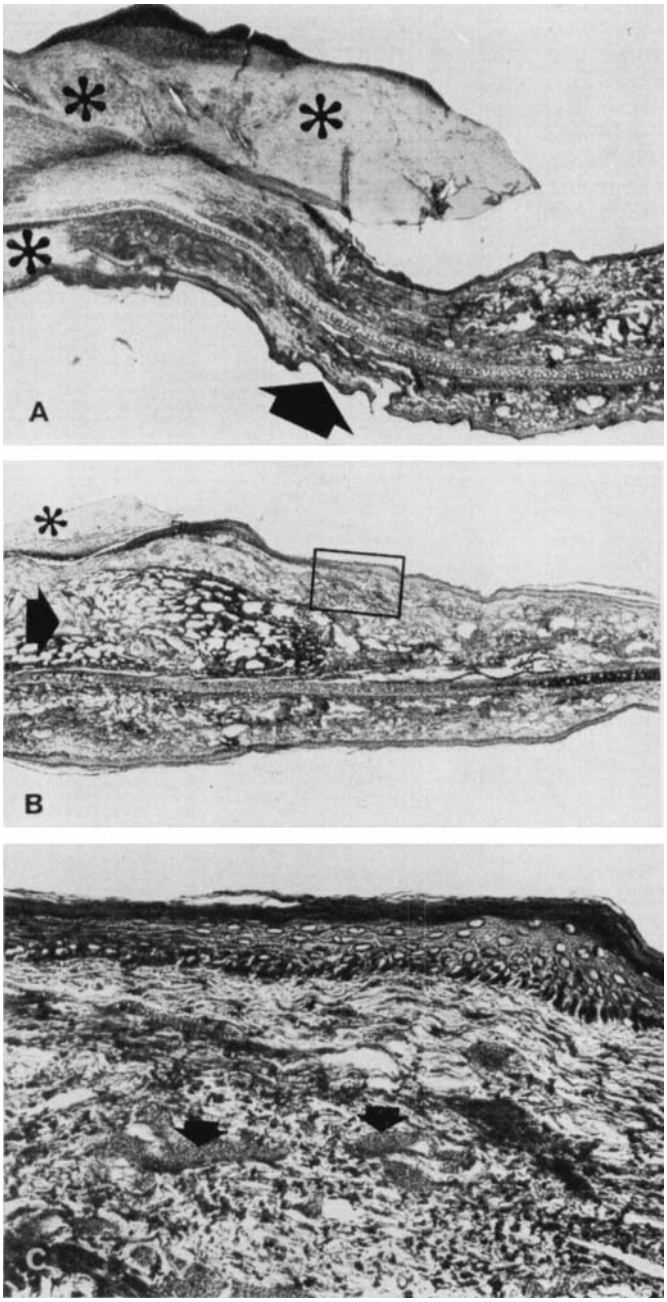

Figure 2. Histology of rabbit ears exposed to different concentrations of MMA monomer. The left part of the pictures represents the centre of the tissue reaction, the right part showing the periphery of the reaction.

A. 100 per cent monomer. Centrally, a large fibrinous crust is seen (*). Peripheral to this, granulation tissue is found (at arrow). Htx-eosin, × 20.

B. 2 per cent monomer. Centrally, a small fibrinous crust is seen at the skin surface (*). Granulation tissue (indicated by arrow) is present below the skin and extends below the cartilage. Htx-eosin, × 40.

C. Detail of B, showing active granulation tissue with round cells and scattered fibroblasts in a loose stroma. Numerous vascular channels are present, two of which are indicated by arrows. The channels appear granular because of their content of barium sulphate. Htx-eosin, × 280.

by the monomer (diameter about 1 cm). A few hours later, an anaemic zone was seen in the centre of this area, the rest of the ear being strongly hyperaemic. By 24 hours, exudation onto the skin had begun from most injection sites. At sacrifice, it was evident that necrosis through the entire thickness of the ear had taken place at the distal injection sites in all cases, whereas, at the proximal injection sites, the skin of the inner surface of the ear usually was intact.

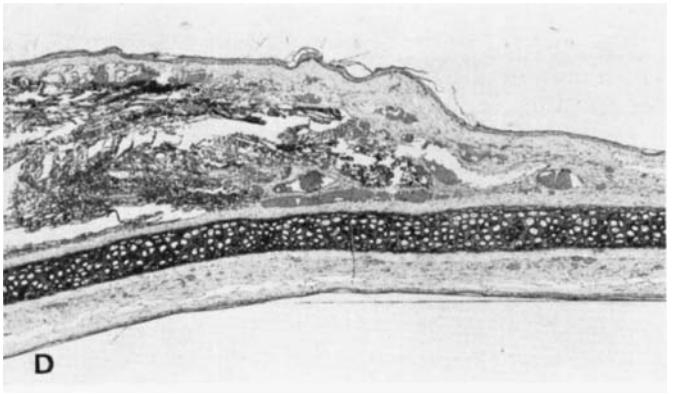

Figure 2 D. 1 per cent monomer. Granulation tissue, not extending below the cartilage, is seen centrally. Htx-eosin, × 40.

The microangiograms revealed a central zone practically devoid of vessels, surrounded by a hypervascularized zone, where a large number of small, newly-formed vessels with irregular topography and varying diameter dominated, i.e. a microvascular picture typical of granulation tissue (Figure 1 A).

Histologically, there was a large central necrotic area, occupied by a fibrinous crust extending through the ear. Bordering this crust was a line of round cells, peripheral to which granulation tissue was found (Figure 2 A). Further away from the centre of the tissue injury a rapid transition to a normal histological picture was noted.

No difference in the tissue reaction of the three monomers was seen.

2 per cent monomer. After injection the mixture stayed in a well-defined, circumscribed area. Macroscopically, the same sequence of events occurred as in the 100 per cent group, only to a far lesser degree. Exudation took place at all injection sites in three out of four animals. In the fourth animal only a local reddening, equally prominent for all monomers, persisted at the end of the experiment.

Microangiographically, a small vessel-free zone with surrounding vascular reaction was seen where exudation had occurred (Figure 1 B); in the other test regions there were vessels even in the central area.

Histologically, where exudation had taken place, a small central fibrinous crust was present above the cartilage. Granulation tissue was found peripheral to this area, and, to a smaller extent, also below the cartilage (Figure 2 B and C). Where exudation did not occur, the skin of the upper surface of the ear was intact and only the area above the cartilage was occupied by granulation tissue.

There was no difference in the reactions of the three monomers.

1 per cent monomer. Macroscopically, only mild reactions were seen. A local reddening was the typical picture. No exudation occurred. In some cases the reddening disappeared during the observation time so that, at the end of the experiment, the area looked normal. However, the disappearance of this reaction always took place several days after that of the unspecific reaction produced by the saline control injections.

Microangiographically, where a reddening was present, a local hypervascularization was seen (Figure 1 C). If the macroscopical reaction had disappeared, a normal vascular architecture was present.

Histologically, where the tissue reaction persisted, granulation tissue was seen above the cartilage. The overlying

skin and the cartilage were normal (Figure 2 D). If the reddening had disappeared, no histological changes were seen in the sections.

In three out of four animals in this group, the reaction caused by the CMW ® and Simplex-P ® monomers disappeared during the observation time, whereas the reaction to the pure MMA monomer disappeared in only one of the same four animals.

0.5 per cent monomer. In no case could a tissue reaction be seen which differed from that produced by the saline control. Microangiographically and histologically, the ears appeared unaffected.

0.03 per cent NNDPT solution. No macroscopical reaction was noted.

Solubility of NNDPT. In two experiments, the solubility of NNDPT in a 1 per cent w/w aqueous solution of pure MMA monomer was found to be 0.058 per cent and 0.047 per cent w/w respectively.

DISCUSSION

The present study confirms all carlier reports that concentrated methyl methacrylate monomer is highly toxic to tissues in contact with it. A fulminant tissue reaction and necrosis always followed its application. However, as the aim of the study was to compare the toxicity of three monomers with different concentrations of N, N-dimethyl-p-toluidine, the reaction produced by the concentrated compounds could not serve as a basis for any conclusions. Surely, differences in toxicity would have been overlooked because of the overwhelming reactions produced. Therefore, the injected doses were lowered to the level of the tissue reaction threshold, since, if the toxicities are the same, the reaction thresholds should also be the same. In order to keep the injection volumes constant, thereby equalizing the mechanical trauma, dilutions of the monomers were made. Isotonic saline was used as vehicle, since it is one of the few solutions available which does not, in itself, cause tissue reaction.

When dilutions of the monomers are discussed, the solubility in water of NNDPT is of interest. The 2 per cent mixture clearly is a suspension of MMA monomer in saline, i.e. a fraction of the monomer is dissolved in the saline and another fraction exists in the form of small droplets in the fluid. The lipid solubility of NNDPT is far greater than its water solubility. Therefore, it is reasonable to assume that the NNDPT will be found mainly in the MMA monomer droplets of the suspension and to a far lesser degree in the water phase. If only the droplets are evenly dispersed, the same proportion of MMA monomer and additives as in the concentrated monomer will be injected.

In the 1 per cent and 0.5 per cent dilutions, the MMA monomer is fully dissolved. In order to ensure an even distribution of the additives in the solution, it is desirable that these should be dissolved together with the MMA monomer. The Simplex-P® monomer has the highest NNDPT concentration of the monomers tested (2.6 per cent), and a 1 per cent solution of Simplex-P ® holds an NNDPT concentration of 0.026 per cent. The study has shown that an NNDPT concentration of approximately 0.05 per cent is obtainable in a 1 per cent MMA solution, and, thus, it is concluded that all components of the monomers tested were fully dissolved in the saline in the 1 per cent and 0.5 per cent groups. The water solubility of NNDPT also means that the accelerator may leak into the tissues from the bone cement, but the clinical implication of this is unknown at the present time.

The results of the study showed that the 2 per cent dilution caused tissue necrosis in all instances. The response to the 1 per cent dilution varied somewhat.

In some cases granulation tissue was formed, indicating a serious tissue injury, but in a number of cases only an unspecific local reaction was noted. The 0.5 per cent dilution in no case caused a registrable reaction. The 1 per cent dilution, thus, seems to correspond to the tissue reaction threshold. However, under other experimental conditions, the reaction threshold may be altered, and, consequently, the absolute figures of the present study cannot be safely used for extrapolation to other animal species or tissues.

The fact that the reaction pattern to the monomers was not uniform in the 1 per cent group can probably be ascribed to methodological errors, as it is logical to assume that such errors have more influence on the magnitude of the tissue reaction at the level of the reaction threshold. It is unlikely that the lack of uniformity was due to differences in monomer toxicity. Two findings support this view: 1) The NNDPT, when tested separately in the same amount as in the 1 per cent Simplex-P ® solution, caused no tissue reaction. 2) The bone cement monomers, which contain NNDPT, caused less strong reactions than the pure MMA monomer.The opposite pattern would have been expected.

On the basis of the data obtained in the present study, it seems reasonable to conclude that the acute local toxicity of the MMA monomer is so high that the possible additional effect produced by NNDPT is overshadowed. This is in good agreement with the conclusions made by Mohr (1958) and Hulliger (1962). For practical purposes, thus, the monomers of the bone cements used today can be considered to be of the same toxicity.

The currently used bone cements have different polymerization peak temperatures and polymerization times (Homsy et al. 1972), and, consequently, may not be equal from a tissue injury point of view. If variations in the acute tissue reaction to the cements are seen, however, it seems that differences in polymerization kinetics rather than in monomer toxicity are responsible.

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Correspondence to: Dr. Lars Linder, Laboratory of Experimental Biology, Department of Anatomy, University of Göteborg, Fack, S–400 33 Göteborg 33, Sweden.