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Experimental studies of the biological response to a new bone cement

Part I *Toxicity of n-butylmethacrylate monomer compared with methylmethacrylate monomer*

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As part of the assessment of the biological properties of a new bone cement (London Hospital cement) which contains poly(ethylmethacrylate) and n-butylmethacrylate, the monomer (n-butylmethacrylate) has been compared in toxicity studies with methylmethacrylate monomer, as used in conventional bone cement. N-butylmethacrylate monomer had an LD_{50} of 1.0 to 1.2 ml kg^{-1} and was therefore only slightly more toxic than methylmethacrylate monomer (LD_{50} 1.2 ml kg^{-1}) when injected intraperitoneally into mice. It did not have any more significant cardiorespiratory depressive effect on intravenous infusion into rabbits, either in single or cumulative doses, when compared with methyl monomer (repeated infusions in 12 rabbits). Both monomers caused a transient bradycardia, tachypnoea, raised central venous pressure and arterial hypotension in low doses with rapid recovery, though the effects were sustained at higher cumulative doses of 60 mg kg^{-1} and above. These results demonstrate that n-butylmethacrylate monomer does not differ significantly in toxicity from the monomer used in conventional cement and can therefore be used as a component in the new material.

1. Introduction

Conventional poly(methylmethacrylate) (PMMA) bone cement has some biological disadvantages. It is known to cause some death of bone at the implantation site which may be related to the heat of polymerization or the effects of monomer [1, 2]. Cardiorespiratory effects at the time of implantation are described in man and experimental animals and considered to be mainly due to the effects of circulating monomer [3–6], although embolism of fat and air to the lung may also play a role [7].

A new bone cement has been developed at the London Hospital. The mechanical properties of this material have been studied at the Imperial College of Science and Technology and have been reported elsewhere [8]. This cement is based upon poly(ethylmethacrylate) polymer powder with n-butylmethacrylate monomer containing 2.5 vol% dimethyl p-toluidine.

Toxicity studies of any new bone cement must include an assessment of the monomer, polymer and of the whole material. In order to evaluate the new material, we have carried out studies on each of these aspects. Results relating to the biological effects of the polymer and the whole material will be presented elsewhere. We wish here to report our studies on the toxicity of n-butylmethacrylate monomer which was assessed by LD_{50} studies in mice, and by experiments on the cardiorespiratory effects of this monomer which were performed in the rabbit by intravenous infusion.

2. Experimental procedure

2.1. Toxicity of monomer injected intraperitoneally into mice

The toxicity of the monomer of the new cement was assessed by intraperitoneal injection into Balb/C mice, recording the numbers of dead animals daily over a seven-day period. Using 20 animals in each group and four groups in each experiment (80 animals on each occasion), a series of preliminary experiments established the approximate dose range on which subsequent studies were based. The percentage of animals dead after 7 days was then recorded in two further experiments over the dose range 1 to 2 ml kg^{-1} body weight using 20 mice per group and five groups. The results were compared with those obtained using methylmethacrylate monomer administered in the same dose range to five groups of 20 mice.

2.2. Toxicity of monomer injected intravenously into rabbits

2.2.1. Methods of anaesthesia

The effects of n-butylmethacrylate were compared with those of methylmethacrylate monomer in two groups of New Zealand white rabbits because different

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anaesthetic agents could affect the results obtained. Seven rabbits (weights 2.2–3.1 kg) were anaesthetized using a 20% solution of Urethane (ethyl carbamate) (May and Baker Ltd) at a dose of 1.5 mg kg^{-1} *iv* while five further rabbits (2.4–3.5 kg) were anaesthetized by continuous intravenous infusion of 0.36–0.38 mg min^{-1} kg^{-1} Althesin (Glaxo Ltd). Although Urethane anaesthesia has a depressant effect upon cardiorespiratory function it has been employed successfully to investigate cardiovascular control in rabbits with cardiovascular pathology [9]. It was used in this study to stimulate the situation of the deeply anaesthetized surgical patient in which adverse effects of either monomer might be compounded by anaesthesia.

2.2.2. Cardiorespiratory monitoring

After anaesthetic induction, femoral and jugular venous catheters were inserted and arterial and central venous pressure measured using calibrated electromanometers, appropriate d.c. amplifiers and a chart recorder. Respiratory rate and end-tidal p_{CO_2} were monitored from the output of a CO_2 analyser (Beckman Ltd). Heart rate was derived from the femoral artery trace and body temperature was monitored and maintained at 37 °C using a rectal thermistor. Experimental infusions of monomer were commenced not less than 30 min after this instrumentation procedure to ensure that the preparation was stable.

2.2.3. Single injections of monomer

In the group of five Althesin-anaesthetized rabbits baseline readings of the monitored parameters were established before intravenous injection over a 1 min period of 1 ml of 10 vol % methylmethacrylate or n-butylmethacrylate monomer in 60/40 alcohol–saline. Control injections of the alcohol–saline vehicle were also included. Each rabbit received control, methyl or n-butyl monomer in a randomized series with sufficient time between injections for the recorded cardiorespiratory parameters to return to control levels.

2.2.4. Cumulative effects of injections of monomer

The cumulative effects of doses of the two monomers were studied in the group of Urethane-anaesthetized rabbits. Single intravenous injections of 15, 30, 60 and 90 mg kg^{-1} of each monomer were given in a stepwise manner sequentially at a minimum of 20 min intervals. Three animals received the methyl and four the n-butyl monomer.

3. Results

3.1. Intraperitoneal injection of monomer in mice

Toxicity tests were carried out in Balb/C mice by the intraperitoneal injection of the monomer of the new cement. Using 20 animals in each of the treatment groups, the numbers of animals dying daily over a seven-day period was recorded. Preliminary experiments showed no effect of n-butylmethacrylate below 0.5 ml kg^{-1}, 5% deaths at 0.7 ml kg^{-1}, 20 and 40% mortality at 1.0 ml kg^{-1} and 95 to 100% death with 2.0 ml kg^{-1} on different occasions. After this series of preliminary studies to determine the appropriate dose range, two separate studies were performed over the range 1 to 2 ml butylmethacrylate monomer per kg body weight. Using five groups of 20 animals. The results were compared with those obtained using methylmethacrylate monomer over the same dose range. The results for one of the seven-day periods for n-butylmethacrylate monomer are shown in Fig. 1. Comparison of the percentages of animals dead at 7 days in the two experiments using butylmethacrylate monomer with those obtained with methylmethacrylate monomer are shown in Table I, from which it may be seen that reproducible results were obtained with the butyl monomer and that this may be more toxic than the methyl monomer at the lower doses in

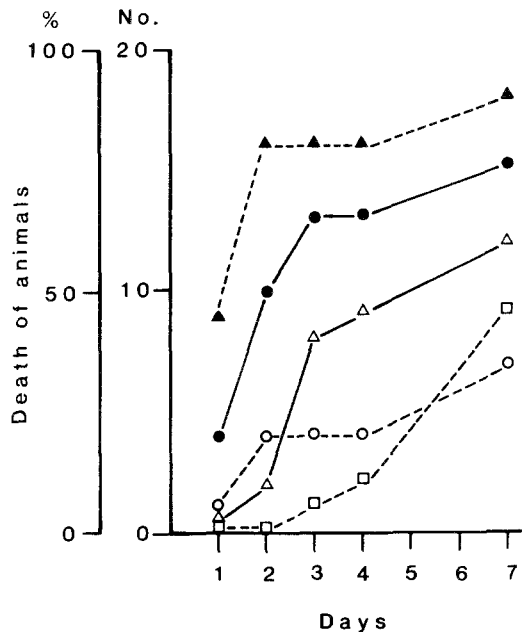

Figure 1 Death of mice (numbers and percentage) over a seven-day period in the dose range 1.0 to 2.0 ml kg^{-1} body weight of n-butylmethacrylate monomer: (□) 1.0, (△) 1.2, (○) 1.5, (●) 1.75, (▲) 2.0 ml kg^{-1}.

TABLE I Percentage of mice dead 7 days after intraperitoneal injection of n-butylmethacrylate in two separate experiments and methylmethacrylate monomer administered for comparison in the stated doses

Dose (ml kg^{-1})	Percentage dead		
	n-Butylmethacrylate monomer		Methylmethacrylate monomer
	Expt 1	Expt 2	
1.00	50	45	20
1.20	60	60	50
1.50	40	35	35
1.75	70	75	75
2.00	80	90	100

the range. The effects at higher concentrations are closely similar between the two materials and although not formally tested in a separate experiment, the LD_{50} for n-butylmethacrylate monomer is shown to be between 1.0 and 1.2 ml kg^{-1}, while that for methylmethacrylate monomer is 1.2 mg kg^{-1}.

3.2. Toxicity of monomers injected intravenously into rabbits

Single intravenous injections of either monomer (0.1 ml monomer in 1 ml total volume of 60/40 alcohol–saline) produced cardiorespiratory changes that were indistinguishable in both nature and magnitude. Increased respiratory frequency and reduced respiratory depth associated with increased end-tidal p_{CO_2} and transient arterial hypotension, bradycardia and increased central venous pressure occurred in response to the injection of either monomer. These changes appeared rapidly during the infusion period but were not sustained once this was complete. All the rabbits given single intravenous infusions survived for a period of not less than 1 h after these injections were complete, regardless of the order in which monomer and control injections had been administered.

The effect of cumulative doses of the two monomers in Urethane-anaesthetized rabbits was also arterial hypotension, raised venous pressure and tachypnoea. The magnitude of the hypotensive response appeared to be dose-related for both monomers. Fig. 2 shows the median blood pressure changes for both monomers (15, 30, 60 mg kg^{-1}). At doses of either monomer above 60 mg kg^{-1}, these parameters did not return to control levels and cardiac arrythmia was evident in each case. Tachypnoea with increased ventilation rate but reduced tidal volume and a markedly elevated end-tidal p_{CO_2} to approximately twice the control level were evident at the lowest dose of either monomer. This respiratory disturbance abated only moderately during the post-infusion period and was further affected by each subsequent infusion. Modest falls of heart rate and elevation of central venous pressure occurred in response to the lower doses of both monomers, but 90 mg kg^{-1} of either monomer was fatal to all rabbits except one that succumbed to the dose of 60 mg kg^{-1} of methylmethacrylate monomer.

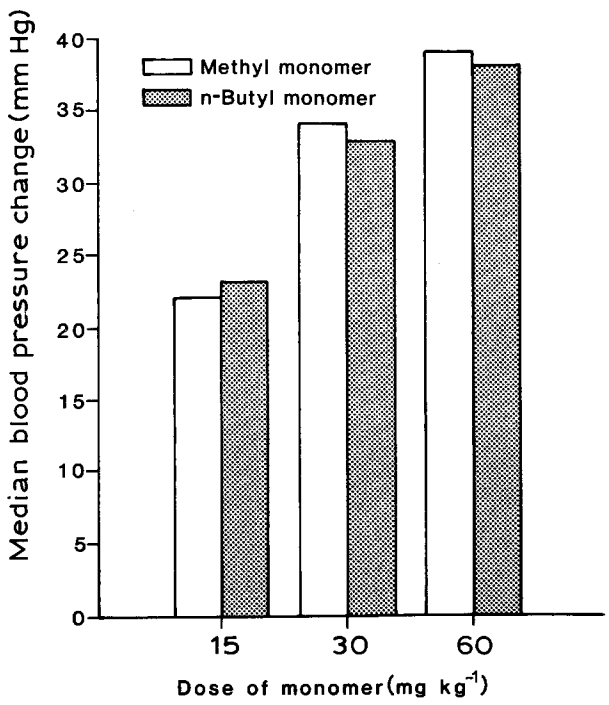

Figure 2 Bar plot showing the median percentage change of mean blood pressure following three cumulative intravenous doses of methyl or butyl methacrylate monomer (15, 30, 60 mg kg^{-1}). Statistical analysis using a non-parametric test showed that the two population changes did not differ significantly at any level of monomer dose.

Each set of results was compared between the two monomer groups using the non-parametric Wald–Wolfowitz runs test. This showed that the changes observed upon monomer infusion were likely to result from a single population. Thus the effects of the two monomers upon cardiorespiratory function did not appear to differ.

4. Discussion

A new bone cement which has some mechanical properties which provide an advantage over conventional bone cement has been developed [8]. It was clearly essential to examine its toxicity in comparison with poly(methylmethacrylate). The new material has some features, such as a low exotherm on polymerization, which may be responsible for its slightly superior biological properties in that it causes less tissue damage and fibrosis on experimental implantation. These effects will be reported elsewhere. The present study is concerned with the toxicity of the monomer which has been compared with methylmethacrylate.

Reproducible results were obtained on intraperitoneal injection of butylmethacrylate monomer into mice and the LD_{50} was found to be between 1 and 1.2 ml kg^{-1}, while that for methylmethacrylate monomer was 1.2 ml kg^{-1}. These findings differ from those of Mir *et al.*, [10] who found butylmethacrylate monomer (isobutyl 1.34 ml kg^{-1}; n-butyl 1.663 ml kg^{-1}) to be marginally less toxic than methylmethacrylate monomer (1.198 ml kg^{-1}). The same authors measured the effect of different methacrylate monomers on isolated perfused rabbit hearts and found that butylmethacrylate had a lesser effect on cardiac rate, force of contraction and coronary flow as compared with methylmethacrylate. They also studied the effects of methacrylate monomers on isolated guinea pig ileum [11], showing that both butyl and methyl monomers elicited a relaxation of smooth muscle and inhibited the contraction responses to acetylcholine and barium chloride. The results obtained were similar with the two monomers. They are consistent with those obtained in the coronary perfusion experiments and suggest that the cardiac effects may be due to a direct vasodilator effect on the coronary circulation.

It is known that bone cements currently used in clinical practice may cause hypotensive episodes during the implantation procedure [3–6] and it had been suggested to us that butylmethacrylate might have cardiorespiratory depressive effects in the rabbit [12]. Our own experience in the toxicity studies in mice

suggested that butylmethacrylate monomer was not significantly different in its toxicity compared with methylmethacrylate monomer. The intravenous infusion of methyl and butyl methacrylates into rabbits produced effects on heart rate, central venous pressure and respiratory function as expected, but these were indistinguishable as between the two agents apart from the hypotension produced by butylmethacrylate monomer which was slightly more severe. Death occurred with respiratory failure, hypotension and cardiac arrhythmias as contributing factors using both monomers.

The levels of monomer administered in these studies were in line with those used for studies of methylmethacrylate monomer in the dog by Ellis and Mullvein [3]. No information is available about the plasma level of butylmethacrylate in humans, but the level of methylmethacrylate after hip replacement has been reported as 2 mg kg^{-1} by Homsy *et al.* [5]. This is a level considerably below that tolerated by all the rabbits in the present experiments (30 mg kg^{-1}). Blood levels of methylmethacrylate monomer fell fairly rapidly until a steady state of 0.02 mg/100 ml was reached 12 min after implantation in dogs, while low levels were reached in 8 min in humans. Homsy *et al.* [5] implanted methylmethacrylate in dogs (dose 1.9 g kg^{-1}) and obtained fairly consistent levels of methyl monomer in the blood, which reached 1.2 mg/100 ml after 2 or 3 min.

Intravenous infusion of monomer caused a catastrophic fall in blood pressure at a dose of 125 mg/100 ml while lower doses (5–25 mg ml^{-1}) had little effect and 50 mg ml^{-1} caused hypotension from which there was recovery in 2 min. The marked hypotensive effects produced by these workers occurred at blood levels one hundred times greater than those reached following implantation procedures in man. Similar results were obtained by McLaughlin *et al.* [13] who showed a peak level of 3.5 mg/100 ml 3 min after implantation of methylmethacrylate 1.3 g kg^{-1} body weight into dogs. Blood levels fell to 0.7 mg/100 ml over the next 16 min.

Taken together, these results suggest to us that PEM/BM is no more toxic experimentally than is PMMA and hence that, since PMMA levels occurring clinically are much less than those required to produce toxicity experimentally, the butyl monomer released from PEM/BM is very unlikely to produce toxic side-effects in man. In this context, it is of note that 19 dogs received intraosseous implants of polymerizing PEM/BM in a study of our own to be reported separately and that none of them showed evidence of cardiorespiratory changes during or after the implantation procedure. Incidentally, no cardiorespiratory problems occurred in 11 dogs receiving PMMA implants, a finding in line with a recent study which showed that there was no evidence that methylmethacrylate monomer was responsible for cardiorespiratory changes when the use of cemented and uncemented femoral implants was compared [7].

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