Silver Polymethyl Methacrylate Antibacterial Bone Cement

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The use of polymethyl methacrylate (PMM) cement has become increasingly more common in implant surgery. Infection in both the immediate and late postoperative periods is a well known complication. In an attempt to lower this infection rate, several types of conventional antibiotics have been added to the cement prior to polymerization[5,6,8,9,10] with promising results. The ideal antibacterial additive should have a broad spectrum of activity, no allergic potential and not affect the mechanical characteristics of the cement. In addition, the antibiotic activity should persist over a long period of time after implantation and not be affected by the exothermic reaction of the polymerizing cement.

Our experience with the antibacterial effects of silver ions[3,4,11,12] suggested that the addition of simple silver salts to the PMM might be an attractive alternative to the conventional organic antibiotics offering maximal effectiveness at low concentrations with low risk.

This article reviews our initial experimentation using 4 silver salts and chlorided silver powder added in various quantities to PMM and tested for bacterial growth inhibition. We were interested in seeing how many of the "ideal" antibiotic characteristics were exhibited by the silver salt additive.

MATERIALS AND METHODS

Four silver salts: 1) silver chloride (AgCl), 2) silver oxide (Ag_2O), 3) silver sulfate (Ag_2SO_4), 4) silver phosphate (Ag_3PO_4) and chlorided silver (Ag-AgCl), all in finely powdered form, were added individually in various quantities to Simplex-P Radiopaque Bone Cement, (Distributed by Howmedica, Inc.). Concentrations of 0.05%, 0.1%, 0.5% and 1.0% of the salts by weight were blended into the dry powder portion of the cement. Small batches of the silver cement (Ag-PMM) powder were polymerized and formed into small tablets using Teflon molds. Each tablet measured 3 mm in thickness and 10 mm in diameter.

To test the antibacterial effectiveness of the various concentrations of the Ag-PMM, the tablets were placed on BHI agar plates which had been inoculated with *Staphylococcus aureus, Pseudomonas aeruginosa* or *Escherichia coli*. After incubation for 24 hours, the diameter of the zone of inhibition of growth around each tablet was measured and recorded for each concentration of each salt. Plain tablets without silver additive were used as controls. All tests were made in duplicate.

An estimate of the longevity of the antibacterial activity of the Ag-PMM was made by bathing the tablets in normal saline solution (10 ml/tablet) to simulate an in vivo situation. The saline baths were kept at 37° and changed every second day. At daily and then weekly intervals the tablets were removed from the solution and placed on agar plates inoculated with *S. aureus* and zones of inhibition were measured after 24 hours incubation (Fig. 1).

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This work was supported by the Veterans Administration R.E.R.D. Service and the Hendrick Research Fund.

Received: December 4, 1978.

0009-921X/79/0900/266 $00.75

TABLE 1. Inhibitory Zone Widths for Ag-PMM Composites

		*Inhibitory Zone Width (mm)**				
	*Bacteria***	*0%*	*0.05%*	*0.1%*	*0.5%*	*1.0%*
	S	0	0	0	0	1
Ag-AgCl	E	0	0	0	0	0.5
	P	0	0	0	0	0.5
	S	0	0	0	0.5	1.5
AgCl	E	0	0	0	0	0.5
	P	0	0	0	1.5	2.5
	S	0	0.5	1.3	4.0	5.0
Ag_2O	E	0	0	0.2	2.0	2.5
	P	0	0.5	3.0	6.0	6.0
	S	0	0.7	0.7	1.8	2.5
Ag_2SO_4	E	0	0.5	1.0	1.8	2.8
	P	0	2.0	2.0	3.0	3.5
	S	0	0	0	0.5	1.5
Ag_3PO_4	E	0	0	0	1.0	2.0
	P	0	0	0.5	2.0	3.5

* Inhibitory zone widths for various composites in 10-mm diameter tablets cultured with bacteria-innoculated BHI agar for 24 hours. Simplex-P, radiopaque bone cement was used.

** S-*Staphylococcus aureus*. E-*Escherichia coli*. P-*Pseudomonas aeruginosa*.

The maximum compressive strength of the Ag-PMM was determined by ASTM standard method D695-77[1] using each of the 1% Ag-PMM combinations and control specimens. Each was mixed and cast into 1.25 cm diameter cylinders and machined to 2.5 cm lengths. An Instron machine was used to conduct the tests.

A preliminary study of the biocompatibility of these composite materials was made by implanting small bars (3 × 2 × 10 mm) cut from polymerized Ag-PMM into the paraspinal muscles of 1-2 kg New Zealand white rabbits in accordance with ASTM standard method F-361-72.[2] The specimens contained 1% (by weight of dry powder portion) of the silver additives and plain PMM bars served as controls. At intervals of 3 days, 2,4,6,8 and 12 weeks, an animal was sacrificed and its 6 implants removed *en bloc* with surrounding muscle and fixed in buffered formalin. After fixation and removal of the implant, the tissue was sectioned and stained for histologic examination.

RESULTS

Antibacterial Effectiveness

The effectiveness of each of the concentrations of the various silver salts, as determined by the growth inhibitory zone width, is shown in Table 1 and illustrated in Figure 1A. The result for each type of bacteria is indicated. Ag_2SO_4 and Ag_2O showed significant inhibition at the 0.05% concentration. Ag_3PO_4 and AgCl had large zones of inhibition only about the tablets with the higher concentrations of the silver salt. Chlorided silver composites seemed least effective. In no case was there a zone of inhibition around any of the control tablets. This series of experiments demonstrated that silver salts do add an antibacterial quality to PMM. Even in low concentrations, Ag_2SO_4 and Ag_2O were effective at inhibiting the growth in vitro of *S. aureus*, *P. aeruginosa* and *E. coli*.

Persistence

As can be seen in Table 2, Ag_2SO_4 was the salt that retained its antibacterial quality longest after

TABLE 2. Persistence of Antibacterial Activity of Ag-PMM vs. *Staphylococcus Aureus* After Normal Saline Equilibrations.

Additive (1%)	*Ineffective After (days)*
Ag-AgCl	1
AgCl	1
Ag_2O	4
Ag_2SO_4	49+
Ag_3PO_4	8

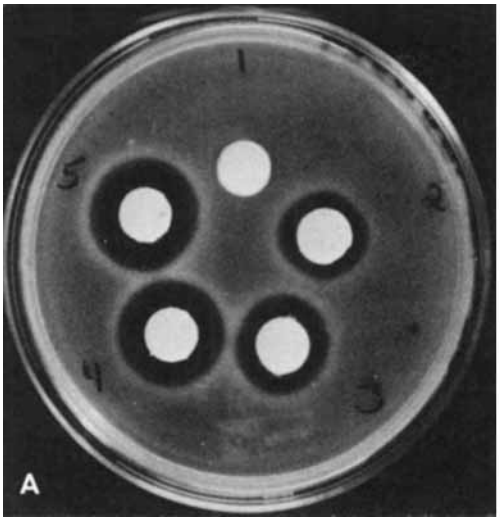


FIG. 1A. Culture plate of *Pseudomonas aeruginosa* showing the inhibitory (dark) zones produced by Ag-PMM tablets containing Ag_2SO_4 in concentrations (by weight of dry powder portion) of (1) 0%, (2) 0.05%, (3) 0.1%, (4) 0.5% and (5) 1%. The tablet diameter is 10 mm and thickness is about 3 mm. Note that the zone width is not linearly related to Ag concentration (see Table 1).

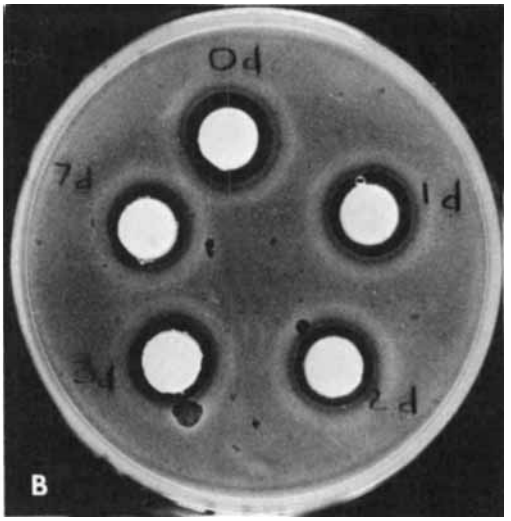


FIG. 1B. *Staphylococcus aureus* culture plate containing Ag-PMM tablets with 1% Ag_2SO_4 which had been equilibrated with several changes of normal saline solution at 37°C for 0 days, 1 day, 2 days, 3 days and 7 days. With this particular additive, clear zones were observed for as long as 7 weeks of saline baths, changed 3 times per week.

exposure to normal saline solution changes. After 7 weeks a small amount of antibacterial activity persisted as indicated by a zone of inhibition about the tablet. All additives seemed to persist in bacterial inhibition for at least 24 hours and those that persisted longest were those that had the higher solubilities (Fig. 1B).

Compressive Strength

The compressive strength of the PMM with the various silver salts is graphically indicated in Figure 2. The average strength for the plain (control) PMM averaged about 82 MPa which is consistent with other reports.[7,9] Only specimens with the highest (1.0%) concentration of silver salt additive were tested for compressive strength. All were in the range of the control specimen with the exception of Ag_2O which seemed to have a reduced compressive strength. This may have been due to the formation of gas bubble inclusions, seen in this material, probably resulting from a reaction during the polymerization process. In general, variation between individual specimens seems to be more a product of mechanical imperfection (air bubbles and scratches) than of the type of silver salt additive. Other mechanical tests (tension, fatigue and shear) remain to be performed and could show differences not evident in compression.

Biocompatibility

Microscopic examination of the tissue surrounding the Ag-PMM coupons showed no evidence of abnormal reactions or deposits and were essentially the same as control specimens of plain PMM. Each implant was surrounded by a thin fibrous capsule containing a few round cells—all consistent with a mild foreign body reaction. Judging from this preliminary evaluation, the capsules tended to be slightly thicker and more cellular in the 2- to 4-week period after implantation (with or without silver) and were quite thin and unremarkable at 8 and 12 weeks (Figure 3).

DISCUSSION

It appears that small quantities of silver combined with PMM add a significant antibacterial quality to the bone cement. The amount of silver salt required is considerably less (one fifth to one

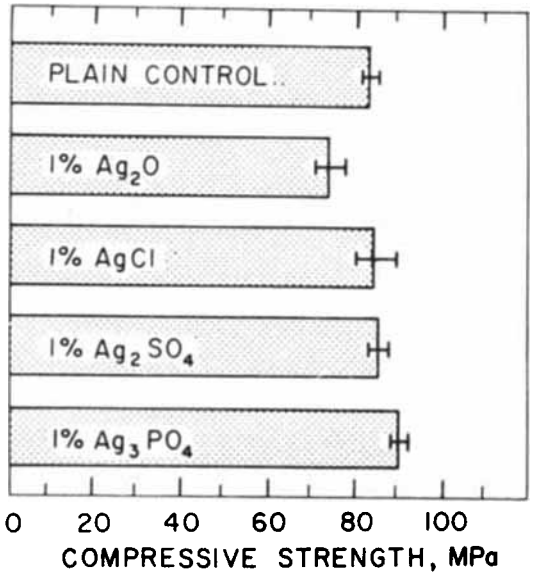


FIG. 2. The maximum compressive strength of replicate specimens (n=6) of silver antibacterial bone cement (Ag-PMM) as compared to controls made of plain cement (Simplex-P, Radiopaque). These tests were performed on the highest concentration of silver compounds used in our experiments (1% w/w dry powder) and thus represents the "worst case." Bars indicate 1 standard deviation. 1 Megapascal (MPa) = 145 pounds per square inch (psi).

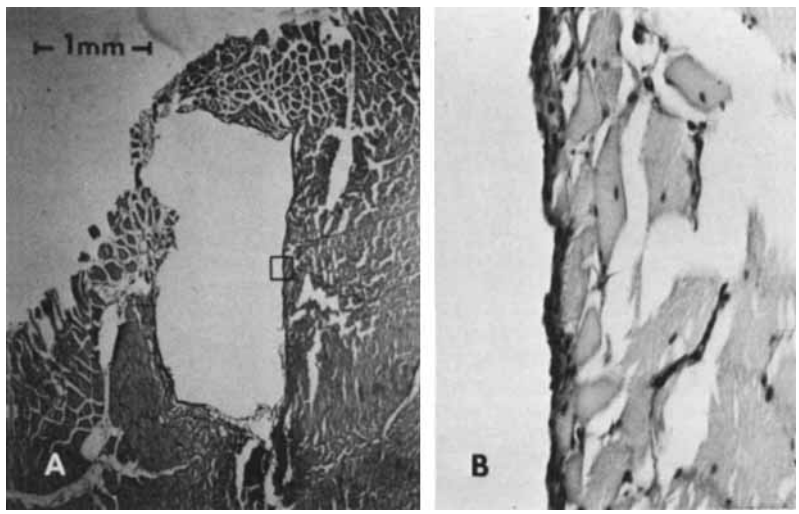
tenth by weight) than for the conventional organic antibiotics. The silver seems to have a number of the qualities of an "ideal" antibacterial additive to PMM. It has a broad range of antibacterial activity[4] and resistance to silver is practically unknown. Although the precise mechanism of action is as yet uncertain, there appears to be little or no toxicity to mammalian tissues at low silver ion concentration. Since the active portion of the silver salt additive is the inorganic silver ion, there should be no change in the antibacterial activity caused by the heat of polymerization of the cement, and none was apparent.

Considering the silver salts tested, Ag_2SO_4 seems to be the most promising for continued investigation. Since significant quantities of SO_4^{-2} are already present in the form of $BaSO_4$ (10%) in radiopaque PMM, essentially all that has been added is 0.017% to 0.34% of silver ions by weight of final cement. Furthermore, the silver in the 0.34% concentration added detectable radiopacity to the barium-loaded cement and its use may serve to reduce the barium required.

In vivo testing and perhaps trials of silver in other types of PMM will be required before the full value of this type of antibacterial additive is known.

SUMMARY

An improved antibacterial bone cement was sought based on the addition of low concentrations of inorganic silver compounds to polymethyl methacrylate. Composites with AgCl, Ag-AgCl, Ag_2O, Ag_2SO_4 and Ag_3PO_4 in concentrations of 0.05% to 1% by weight, were tested in vitro against bacterial cultures. All were effec-



FIGS. 3A and B. (A, left) Histologic appearance of tissue surrounding Ag-PMM implant in rabbit paraspinal muscle after 12 weeks in situ. Ag_2SO_4 (1% w/w). (B, right) 10× magnification of indicated region in A. The thin membrane and lack of reactivity was typical of the other additives tested as well as plain PMM control implants.

tive, but Ag_2SO_4 was especially so, even after 7 weeks of incubation in normal saline. Compressive strength of the cement was not affected by these additions, except in the case of Ag_2O. Biocompatibility tests in rabbit muscle for up to 12 weeks showed no significant difference between the Ag-PMM and plain PMM in tissue reactivity, both being minimal. These features, coupled with the broad spectrum of antibacterial activity and low allergic potential of silver, make Ag-PMM an attractive alternative to conventional organic antibiotic/bone cement composites.

ACKNOWLEDGEMENT

We are very grateful to Ms. Sharon Chapin, Mr. Fred Werner and Dr. Hansen Yuan for their assistance in these experiments. This work was supported by the Verterans Administration R.E.R.D. Service and by the Hendricks Research Fund.

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