Evaluating suppression of PGE2, PAF, and histamine synthesis and histopathological changes of bones in the membrane surrounding particulate polymethylmethacrylate in the rat tibia

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Abstract

Introduction: Inflammation and wear debris may be responsible for bone lysis and subsequent lost in aseptic arthroplasty. Prostaglandin E2 (PGE2), platelet activating factor (PAF), and histamine are important mediators of inflammatory cells. We studied histopathological changes of cement-bone interface after using specific antagonists of these mediators.

Methods: Left and right tibiae of 120 rats in ten groups were drilled. The left side was filled with polymethylmethacrylate (PMMA) and the right side was used as control. The first three groups respectively received 1mg/kg, 10mg/kg, and 25mg/kg of terfenadine, the second three groups respectively received 0.08mg/kg, 0.32mg/kg, and 0.64mg/kg of alprazolam, and the third three groups respectively received 1mg/kg, 5mg/kg, and 25mg/kg of naproxen. The tenth group received no drug and served as the control group. The animals were sacrificed after 16 weeks and studied by one pathologist.

Results: Cellular reaction in the left side was significantly more than the right side in all cases. Medium and high doses of terfenadine and naproxen and high doses of alprazolam could also significantly decrease giant cells and histiocytes.

Conclusion: Increased cellular reaction in the cement-bone interface was suppressed by administration of PGE2, PAF, and histamine specific inhibitors. The use of these agents may induce retardation of the bone loss associated with early prosthetic loosening.

Keywords

PMMA, arthroplasty, aseptic loosening, PGE2, PAF, histamine, terfenadine, alprazolam, naproxen, histiocyte, giant cell.

Introduction

Even though bone cement is considered to be the weak mechanical link in the prosthetic construct, PMMA is a frequent material to stabilize prosthetic joints within bone. Possible cement fractures due to intermittent loading of cemented joints will result in generation of cement debris, while this debris may cause aseptic loosening of the joints. The mechanism is thought to be stimulation of an aggressive fibrohistiocytic and giant cell interfacial membrane caused by the debris[1-11]. It was demonstrated that particulate PMMA evokes a florid foreign body response composed of histiocytes and giant cells surrounding and engulfing cement debris[12]. The membrane surrounding particulate PMMA can produces significant amounts of PGE2, tu-

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mor necrosing factor (TNF), interleukin [1,6,8], platelet activating factor (PAF), and other substances capable of stimulating bone lysis [2-4,8,14].

As mentioned in previous studies, high PGE2 values are produced in tissue culture by the membrane surrounding particulate cement polymer [2,12-15]. These mediators' specific antagonists may be indicated in retarding the bone loss associated with arthroplasty loosening. Studies have been performed to understand the role of these mediators' antagonists to prevent early prosthetic loosening; thus, the use of non-steroidal anti-inflammatory drugs has been suggested2. However, the role of some of these antagonists in preventing early prosthetic loosening is not completely identified yet.

The goal of this study was to determine whether the increased PGE2, PAF, and histamine values from the membrane surrounding particulate cement implants could be decreased by administration of their specific antagonists (naproxen, alprazolam, and terfenadine respectively).

Methods

120 white-male-wistar rats weighing about 250gr were used in the study. A standardized implantation technique was followed [2,12, 16,17]. Using sterile technique and after general anesthesia by intra-peritoneal administration of 5mg/kg of xylocaine and 50mg/kg of ketamine, a 2mm drill hole was made in the tibia, entering the medullary canal of the tibia, approximately 1cm distal to the knee joint bilaterally. Afterward, the marrow of the medullary canal was suctioned below the drill hole. In each case, the cavity in the left tibia was filled with CMW1 orthopedic bone cement (DePuy CMW, Blackpool, England) and the right leg was used as a prepared, but non-implanted, control. CMW1 consists of both spherical and comminuted particles (irregular in shape) ranging in the size from 5 to 75 μm with 9.1 wt% barium sulfate. The animals were fed a standard diet.

The effect of various antagonists with different doses was investigated by dividing the 120 rats into ten groups of 12 animals. The first three groups respectively received 1mg/kg, 10mg/kg, and 25mg/kg of terfenadine, the second three groups respectively received 0.08 mg/kg, 0.32mg/kg, and 0.64mg/kg of alprazolam, and the third three groups respectively received 1mg/kg, 5mg/kg, and 25mg/kg of naproxen, while the tenth group received no drug and served as the control group. Doses of the inhibitors were chosen with the help of a pharmacologist and using the previous publications[18-25].

Sixteen weeks after implantation, the animals were sacrificed thorough a barbiturate overdose under anesthesia using Xylocaine and Ketamine. The lower extremities of the animals were prepared to expose the legs. The implant area in the tibia was harvested and the drill-hole site meticulously extracted with a curette. Medullary canal contents were harvested from the right and the left tibia in each animal with the same volume and the samples were sent to the pathologist.

The tissue samples were fixated and decalcified. Subsequently, hematoxylin and eosin stained sections were retrieved and examined by an experienced pathologist, who was not aware of the experiment. Another pathologist studied random samples of each group to test the precision and accuracy of the first pathologist. Inflammatory cell counts were assessed by randomly counting 10 different high power microscopic fields (×400 magnification) and recorded as the number of inflammatory cells/10 high power fields (HPF). These cells were classified into groups of giant cell, histiocyte, polymorphonuclear (PMN), lymphocyte, plasma cell, and fibrocyte.

Statistical analysis was accomplished by performing the independent t-test between right and left sides within each group and then the same test was performed to compare the left

Studied Cells	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Control Group
Giant	3.1±0.8	2.2±1	2.1±1.3	4.1±0.8	3.4±0.6	2.1±0.8	2.7±0.6	2±0.8	0.8±0.1	3.8±0.8
	0	0	0	0.2±0.1	0	0	0	0	0	0.3±0.1
Histio	4.2±1.2	2.1±0.3	1.8±0.1	4±1.3	3.9±1.2	2.1±0.7	3.9±1.8	2.8±0.7	1.9±0.5	4.1±1.2
	1.4±1.1	1.5±0.1	1.1±0.3	1.1±0.1	0.9±0.2	0.8±0.1	1.9±0.8	1.4±0.6	0.9±0.1	1.6±0.4
PMN	0.3±0.1	0.1	0	0.5±0.1	0.5±0.1	0.2±0.1	0.2±0.1	0.3	0.1	0.5±0.1
	0.1	0.1	0	0.2±0.1	0.3±0.2	0	0.2±0.1	0.1	0	0.4±0.1
Plasma	1.2±0.1	0.8±0.1	0.3±0.2	1.2±0.1	0.9±0.5	4.5±0.5	1.1	0.8	0.4	1.3±0.1
	0.1	0	0.1	0.1±0.1	0.2	0	0	0.1	0	0.3
Lymph	14±2	11±2	9.1±1.4	16±2	15±2	5.8±1.2	13±1	10±1	4±0.7	15±2
	2.1±1.2	2.5±1.4	1.5±0.8	2.5±0.6	3±0.8	2.3±0.8	3.9±2.1	3.1±1.3	2.1±0.1	4.7±1.2
Fibro	78±15	40±10	10±1	83±11	76±19	41±10	73±20	50±11	10±5	83±9
	0	3.1±0.1	0	0	0	0	0	0	0	0.2

* Gray lines specify values of the left tibia, while values of the right tibia are shown in white lines.

Table 1. The mean and SD of inflammatory cells in left and right tibiae of all groups.

sides with the control group (tenth group). When comparing more than two groups, a one-way analysis of variance (ANOVA) was performed to determine which groups differed significantly. The data were analyzed with the SPSS statistical software (SPSS Version 15, Chicago, IL, USA), and the level of significance was set at p value < 0.05.

Results

Of 120 rats used in this study, four animals died from no apparent cause. One animal from group 5 died after two months and the rest died in the third month, respectively from group 2, 7, and 10. There were no other complications noted in the remaining rats in this study. All animals except two of them (ambulated after 3 days) began ambulating within several hours of surgery, and all wounds healed without incident.

The mean and standard deviation of inflammatory cells in left and right tibiae of all groups are demonstrated in Table 1. The right tibia was used as a prepared, but non-implanted, control and the tenth group received no drug and served as the control group. Values of giant cells and histiocytes between left and right tibiae in each group were compared. In all groups, a significant difference (p value < 0.001) between left and right tibiae was seen. Comparison of values for giant cells and histiocytes between group 1

(that had received 1mg/kg of terfenadine) and the control group (group 10) showed no significant difference. Differences between values of giant cells in both groups 2 and 3 (that had received respectively 10mg/kg and 25mg/kg of terfenadine) and the control group were statistically significant (p values < 0.01); Moreover, values of histiocytes in the recent groups (2 and 3) in comparison with the control group, demonstrated significant differences with lower p values (p values < 0.001). Comparison of giant cells and histiocytes between both groups 4 and 5 (that had received respectively 0.08 mg/kg and 0.32mg/kg of alprazolam) and the control group revealed no significant differences. Differences between values of giant cells and histiocytes in group 6 (that had received 0.64mg/kg of alprazolam) and the control group were significant (p values < 0.001). Values of giant cells in group 7 (that had received 1mg/kg of naproxen) in comparison with the control group, showed a significant difference (p value = 0.02); while histiocytes in this group, in comparison with the control group, demonstrated no significant difference. Comparison of giant cells and histiocytes in group 8 (that had received 5mg/kg of naproxen), with the control group, revealed significant differences (p values < 0.01). As a final point, differences between values of giant cells and histiocytes in group 9 (that had received 25mg/kg of naprox-
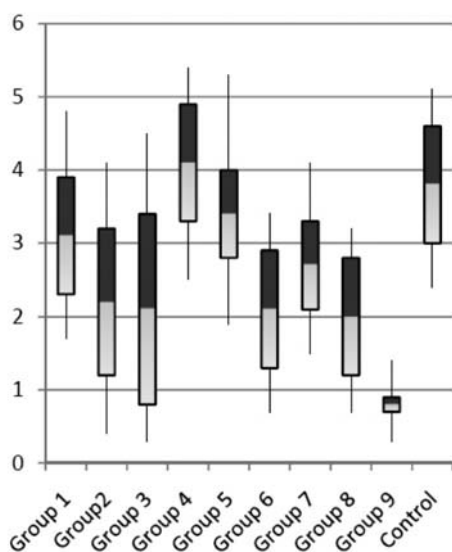
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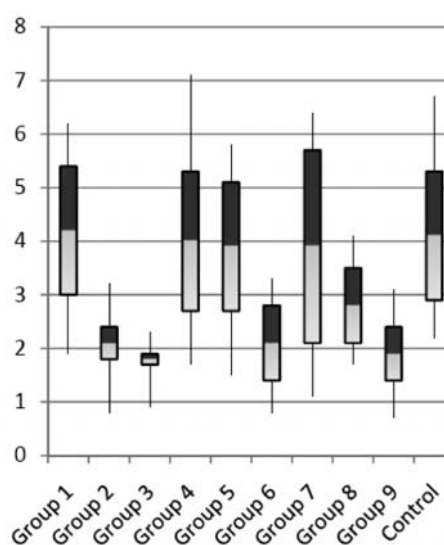Fig. 1. Distribution of giant cells among different groups.

Fig. 2. Distribution of histiocytes among different groups

en) in comparison with the control group, showed statistically significances (p values < 0.001). Distributions of giant cells and histiocytes among different groups are shown in Figs 1&2.

Discussion

The biologic mechanisms of aseptic loosening of cemented arthroplasties are poorly understood. In patients with aseptic loosening, the tissue response at the interface between cemented prosthesis and bone is characterized by the infiltration of cells associated with chronic inflammation and by the loss of bone (i.e., osteolysis). Triggers for accelerating this inflammation and cells or cell products, which are responsible for bony destruction, are not clearly known yet. Previous histologic studies have shown that cement polymer evokes a fibro-histiocytic and giant cell reaction similar to that seen surrounding loose joint arthroplasties in humans [16]. In these studies, the immediate interface between bulk cement implants and bone was composed of mature fibrous tissue and contained only 5% - 10% histiocytes and giant cells, while surrounding particulate cement

polymer was composed of 25% - 30% histiocytic and giant cells16. In addition, in some studies, increasing PGE2 production by the membrane surrounding loose cemented prostheses and a more florid foreign body reaction in humans compared to non-loosed prostheses has been demonstrated [3,4,8,26,27].

Foreign body giant cells and histiocytes have been shown to phagocytose small cement particles but are unable to digest them [16,28]. The indigestible cement particles and substances such as PGE2 are egested from the phagosomes and released into the extracellular compartment28. Elevated PGE2 levels have been shown to cause increased bone resorption in vivo and in vitro [29,30]. Particulate cement debris may play an important role in the process of arthroplasty loosening by stimulating production of inflammatory cell mediators (i.e., PGE2, PAF, and histamine) and following periprosthetic bone lysis [28,31]. The effect of administration of a cyclo-oxygenase inhibitor was investigated in previous studies and it was shown that the membrane surrounding particulate cement polymer produced lower contents of PGE2 due to administration of specific antagonists

[2]. Despite the fact that elevated levels of PGE2 have been suppressed by administration of specific antagonists, it is not known whether the inhibition of other inflammatory mediators like PAF and histamine would have the same effect.

Our study supports the findings of previous studies that in comparison between the site of implanting cement polymer and the prepared, but non-implanted control, giant cells, histiocytes, and other inflammatory cells were increased in the interface between cement implants and bone. The present study has demonstrated that the increased giant cells and histiocytes in the place of implantation could be decreased by different doses of specific antagonists of PGE2, PAF, and histamine. Using medium and high doses of naproxen, high doses of alprazolam, and medium and high doses of terfenadine that are respectively specific antagonists of PGE2, PAF, and histamine, could suppress the giant cells and histiocytes in the membrane surrounding particulate cement polymer. Low doses of naproxen suppressed only giant cells, but it could not suppress histiocytes. Furthermore, low and medium doses of alprazolam, and low doses of terfenadine could not suppress giant cells or histiocytes.

The cemented joint arthroplasty may experience aseptic loosening, a complex phenomenon involving mechanical and biologic factors and the particulate debris appeared to play an important role in this process. Previous studies in humans have implicated inflammation as a possible biological mechanism stimulating the bone lysis associated with prosthetic loosening [2,3,9]. Specific antagonists of PGE2, PAF, and histamine are known to inhibit the inflammation in the membrane surrounding particulate cement polymer [2,12,13]. Future animal studies are necessary to assess whether the administration of these drugs could prolong the lifetime of cemented and cement-less prostheses with early signs of prosthetic loosening.

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