ACRYLIC BONE CEMENTS INDUCE PLATELET ACTIVATION AND RELEASE OF TRANSFORMING GROWTH FACTOR BETA

*Baldini, N; +*Cenni, E; *Ciapetti, G; *Savario, L; *Granchi, D; *Giunti, A
+*Laboratory for Pathophysiology of Orthopaedic Implants & Department of Orthopaedic Surgery, Rizzoli Institute, Bologna, Italy. +*Fax: +39,(0)51.6366896, Fax: +39,(0)51.6366748, elisabetta.cenni@ior.it

INTRODUCTION
During total joint replacement procedures, prosthetic devices get in direct contact with circulating blood, and a rapid activation of platelets and of the coagulation cascade occurs. In cemented prostheses, methylmethacrylate monomer (MMA) is released into the bloodstream from curing cement, and may therefore cause local and systemic effects. Data on the effects of bone cements on platelets, however, are very scanty. Based on the hypothesis that MMA might also have effects on platelets (1), we have investigated the platelet release reaction after in vitro contact with a number of commercially available bone cements. Among the products of platelet release reaction, beta-thromboglobulin (β-TG), that specifically indicates platelet activation, and transforming growth factor-beta 1 (TGF-β1), that is also produced by other cell types, but that is known to have a specific action on bone, were assayed.

MATERIALS AND METHODS
Seven acrylic bone cements were evaluated: Cemex Rx® (Tecres, Italy), Cemex Isoplastic® (Tecres), a bone cement at low viscosity (Zimmer, IN), bone cement, dough type (Zimmer), CMW 3® (DePuy, England), Cerim LT® (Cremascoli, Italy), and Palacos R® (Merck, Wehreim, Germany). The powder and the liquid components of each cement were mixed according to the manufacturer’s instructions. After polymerization, each cement was allowed to cure for 15 minutes, and then put in contact in vitro with human plasma enriched in platelets. Plasma in contact only with siliconized glass was used as a negative control. All the samples were placed in a mixer for 30 minutes at room temperature. Immediately after contact, the number of platelets was determined on an aliquot of each sample. The other aliquot was centrifuged for 45 minutes at 2000 g at 4° C. On the obtained platelet-deprived plasma, β-TG and TGF-β1 were assayed by enzyme immunoassays (Asserachrom beta-TG, Diagnostica Stago; Human TGF-beta 1 ELISA, Bender Med System, Austria). Descriptive data were expressed as mean±standard error. Statistical evaluation of the material effects in comparison with negative controls was carried out using the Student’s paired t-test. The correlation between cement composition and platelet parameters was analyzed by calculating the Spearman’s ρ coefficient. P≤0.05 was considered statistically significant.

RESULTS
Ten separate experiments were performed for each biomaterial. The bone cement at low viscosity from Zimmer induced a significant decrease of platelet number compared with the negative control (149,600±36,940 platelets/µl vs. 396,100±15,790 platelets/µl; p<0.01). A less significant decrease was also determined by Palacos R® (352,400±21,510 platelets/µl; p<0.05). No significant change in the platelet number was induced by other bone cements. Among the two cements that induced the greatest platelet number decrease and release reaction, as shown by the β-TG increase, Palacos R® induced also TGF-β1 increase. Only CMW 3®, the cement at low viscosity from Zimmer, and Palacos R® induced also TGF-β1 increase. The observed discrepancy between β-TG and TGF-β1 could be linked to a higher sensitivity of β-TG assay in the release reaction evaluation. The greatest platelet modifications were determined by Palacos R®, which was the only cement with zirconium dioxide. The cements with the highest contents of barium sulphate and benzoyl peroxide induced the lowest TGF-β1 release.

In other studies, the extracts of the cement at low viscosity from Zimmer, but not Palacos R®, were cytotoxic to L929 cells (2). Cerim LT®, which was highly cytotoxic, had no effect on platelets. Among the two cements that induced the greatest platelet activation, only Palacos R® had an apoptotic effect on lymphocytes (3). This suggests that the effect on platelets is linked neither to the cytotoxicity nor to the apoptotic activity of cements.

Our findings demonstrate that cements induce platelet release, as shown by significantly increased levels of β-TG. CMW 3®, Palacos R®, and the cement at low viscosity from Zimmer brought about a highly significant increase (p<0.01) in TGF-β1 compared to the negative control (negative control 5.79±0.29 ng/ml; CMW 3®: 8.19±0.73 ng/ml; Palacos R®: 17.7±2.73 ng/ml; cement at low viscosity: 18.13±1.31). The other bone cements induced no significant modifications of TGF-β1 (Cemex Rx®: 5.81±0.36 ng/ml; Cemex Isoplastic®: 5.81±0.30 ng/ml; cement-dough type: 7.38±0.91 ng/ml; Cerim LT®: 6.71±0.48 ng/ml). A positive significant correlation was found between the platelet number and the concentration of benzoylperoxide in the cements (r = 0.429). A significant negative correlation was found between β-TG and the levels of methylmethacrylate monomer (r = -0.265) and benzoylperoxide (r = -0.426). A significant negative correlation was found between TGF-β1 and the levels of methylmethacrylate monomer (r = -0.570), benzoylperoxide (r = -0.405), and barium sulphate (r = -0.166).

DISCUSSION
The decreased number of platelets detected in samples exposed to cements compared with the negative control could be caused by platelet adhesion to the biomaterial. The cements at low viscosity from Zimmer and Palacos R® induced both the greatest platelet number decrease and release reaction, with highly significant increases of β-TG and TGF-β1. None of the other cements determined platelet adhesion, but all induced a significant release reaction, as shown by the β-TG increase. Only CMW 3®, the cement at low viscosity from Zimmer, and Palacos R® induced also TGF-β1 increase. The observed discrepancy between β-TG and TGF-β1 could be linked to a higher sensitivity of β-TG assay in the release reaction evaluation. The greatest platelet modifications were determined by Palacos R®, which was the only cement with zirconium dioxide. The cements with the highest contents of barium sulphate and benzoyl peroxide induced the lowest TGF-β1 release. In other studies, the extracts of the cement at low viscosity from Zimmer, but not Palacos R®, were cytotoxic to L929 cells (2). Cerim LT®, which was highly cytotoxic, had no effect on platelets. Among the two cements that induced the greatest platelet activation, only Palacos R® had an apoptotic effect on lymphocytes (3). This suggests that the effect on platelets is linked neither to the cytotoxicity nor to the apoptotic activity of cements.

Our findings demonstrate that cements induce platelet release, as shown by significantly increased levels of β-TG. CMW 3®, Palacos R®, and the cement at low viscosity from Zimmer determined also a significant secretion of TGF-β1. Platelet activation can contribute to the pathogenesis of deep venous thrombosis after prosthetic surgery.

REFERENCES