Activation of the complement system and adverse effects of biodegradable pins of polylactic acid (Biofix®) in osteochondritis dissecans

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Biodegradable pins of polyglycolic acid (PGA) or polylactic acid (PLA) have been used in the treatment of fractures and osteotomies during the past 5 years. Adverse effects reported have included swelling at the implantation site and sinus formation, considered to represent nonspecific foreign-body reactions. Recent reports, however, have shown severe reactions after intraarticular fracture fixation. Reactions in 2 patients, treated with polylactic pins for osteochondritis dissecans (OCD) in our hospital, prompted the present clinical investigation and further evaluation of the complement-activating potential of polylactic pins.

10 knees underwent arthroscopic fixation of an OCD-lesion with Biofix® (PLA) pins. Clinical follow-ups were carried out at 2, 6, and 12 weeks and at 6 and 12 months. Blood samples were collected from 5 patients 9–24 months postoperatively for biocompatibility tests. Quantification of human C5a des Arg was performed with a recently developed sandwich ELISA technique, using neoepitope-specific monoclonal antibodies.

6 knees developed diffuse swelling and a prolonged postoperative course. 2 patients had a particularly prolonged course which could not be attributed to infection. Levels of C5a des Arg in plasma incubated in the presence of polylactic acid were higher than in plasma incubated in the absence of PLA. The high frequency of long-term postoperative inflammatory signs in these knees treated for OCD and the demonstration of a complement activation potential of PLA pins warrant further studies on the biocompatibility of this material. Until more information is available, we do not recommend intraarticular use of PLA pins.

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Biodegradable pins of polyglycolic acid (PGA) or polylactide (PLA), both Biofix®, have been used in the treatment of fractures and osteotomies during the past 5 years (Böstman et al. 1989, 1990). The adverse effects reported have been swelling at the implantation site and sinus formation after an average of 12 weeks in approximately 8 percent of the patients (Böstman et al 1990). This has been explained as a typical nonspecific foreign-body reaction. Recent reports have shown that this material may not be biologically inert (Casteleyn et al. 1992, Frøkjær and Møller 1992). The most severe reactions seem to occur after intraarticular fracture fixation (Barfod and Svendsen 1992, Fridén and Rydholm 1992).

The report from Fridén and Rydholm (1992) of a severe aseptic synovitis of the knee after fixation of an osteochondral lesion with Biofix® pins, triggered a clinical evaluation of our patients and further evaluation of the complement-activating potential of Biofix® pins.

Activation of plasma complement has been considered a major mechanism by which devices coming into contact with blood may cause harmful effects to the host, e.g., membranes used for hemodialysis or extracorporeal circulation (Jørstad 1987). During activation of the complement system, proinflammatory peptides, termed anaphylatoxins, are enzymatically cleaved from parent complement components and released. Of these, C5a is the biologically most potent and is capable of inducing a series of both humoral and cellular proinflammatory responses, reviewed by Hugli (1984). Consequently, detection of the complement-activating potential of blood-contacting materials is becoming a more widely used parameter of biocompatibility (Chenoweth 1987). Here we report the capability of Biofix® pins to activate the complement system in vitro by measuring the generation of anaphylatoxin C5a des Arg, the plasma analogue of C5a.

Patients and methods

9 patients (10 knees) with no history of inflammatory joint disease underwent arthroscopic fixation of large, symptomatic osteochondral fragments using from 2 to 5 Biofix® pins. There were 5 men and 4 women, with an average age of 19 (12–47) years. Postoperatively the knees were immobilized in a cast (1) or orthosis (9) for 6 weeks. Weight bearing as tolerated was allowed after 4 weeks. Clinical follow-ups were carried out at 2, 6, and 12 weeks, and at 6 and 12 months. From 5 patients we collected blood samples on average 16 months postoperatively for biocompatibility tests.

Quantification of C5a des Arg

Quantification of human C5a des Arg was performed by a recently developed sandwich ELISA using neoepitope-specific monoclonal antibodies (MAbs) (Bergh and Iversen 1992). This assay is far more sensitive than the commercially available radioimmunoassay (RIA) manufactured by Amersham (Wagner and Hugli 1984), and, more important, unlike the RIA method it may be performed directly on plasma or serum samples as the presence of native complement component C5 will not interfere with the results obtained. The ELISA procedure for quantification of C5a des Arg has been described in detail elsewhere (Bergh and Iversen 1992). Briefly, microwells of Nunc Immunoplates Maxisorp (Nunc, Roskilde, Denmark) were coated with MAb 4A2E10E2 in 0.1 M carbonate buffer, pH 9.6, by overnight incubation at 20 °C. Unoccupied binding sites were blocked by incubating the wells with 1% skimmed milk in phosphate-buffered saline, pH 7.2, (PBS) for 30 min. Human serum or plasma in triplicate diluted in PBS containing 0.05 % Tween-20 (Sigma Chemical Co., St. Louis, MO) (PBS-T) was then incubated for 1.5 h and washed with PBS-T. Biotinylated MAb 3G3C4 in PBS-T was then incubated for 1 h, followed by 3 washes with PBS-T. Peroxidase-conjugated streptavidin was then incubated for 1 h. After washing, substrate ortho-phenylene-diamine (OPD) and H_2O_2 were added. The enzymatic reaction was terminated by adding 2 M H_2S0_4 and the optical density (OD) at 492 nm was read. The concentration of C5a des Arg in plasma or serum was determined by relating the OD to a standard curve obtained by employing known concentrations of C5a des Arg.

Complement activation of Biofix® pins, experimental design

As complement source we used heparinized plasma or serum obtained from 5 patients in whom Biofix® pins were implanted 9–26 months prior to blood sampling, and from 3 healthy staff members.

PLA pins, diameter 2.0 mm (Biofix®) were cut into 6–8 fragments in the operating theater using sterile, autoclaved instruments. Care was taken not to touch the pin or fragments with gloves, and the fragments were transferred to the plasma using a sterile forceps. Fragments derived from one pin were placed in 1 mL plasma in a sterile, siliconized tube (Becton Dickinson, Meylan Cedex, France) and the tube was incubated at 35 °C for 2 h while being gently rolled. 1 mL of plasma incubated under identical conditions in the absence of pins served as control. After 2 h, any further complement activation was terminated by adding 1 mL of 20 mM ethylenediaminetetraacetic acid (EDTA) in 0.9% saline. The samples were frozen at –80 °C for subsequent C5a analyses. In order to study the effect of adsorption of plasma proteins on the complement-activating potential of the pins, plasma was removed after the initial 2 h incubation and replenished with homologous fresh plasma or serum and incubated further.

Results

The average follow-up was 10 (3–22) months. In 5 knees the postoperative course was uneventful. 3 knees had a diffuse swelling and prolonged postoperative course with limited range of motion for 3 months. All 3 had normal clinical findings after 6 months. 2 knees developed moderate to large effusion and other signs of inflammation postoperatively. Case 4 was a 47-year-old woman who was doing well after 4 weeks but developed knee pain, swelling, warmth and limited range of motion at 7 weeks. Infection and deep vein thrombosis were ruled out. Her symptoms have persisted 10 months postoperatively. She has a moderate effusion, knee pain, and reduced range of motion. Case 1 was a 12-year-old boy who 3 days postoperatively developed a severe inflammatory reaction. Several bacterial cultures from the joint fluid were negative. The analysis showed a massive number of polymorphonuclear leukocytes (PMN) in the joint fluid. Blood samples showed an ESR of 60, CRP 45 and WBC 11.4. The patient was treated with intravenous antibiotics. 8 weeks postoperatively, he still had effusion and limited range of motion, but the ESR had decreased to 5

Table 1. Effect of polylactic acid pins on the generation of C5a in plasma

		C5a des Arg (ng/mL)	
		Plasma control	Plasma + polylactic acid pins
Patients	1	19	32
	2	12	35
	3	29	52
	4	15	26
	5	12	20
Staff	6	28	42
	7	16	22
	8	33	42

and CRP was less than 5. At 9 months he had normal clinical findings.

Complement-activating potential of polylactic acid pins

In each of the 8 plasmas investigated, the presence of polylactic acid pins during incubation resulted in an increase, mean 13 (6.4–23) ng/mL, of the C5a des Arg concentration as compared to control plasma ($P < 0.001$ paired Student's t-test; Table 1). The polylactic acid-associated increase in C5a des Arg was observed in plasma from the patients as well as from members of the staff.

In 7 of 8 plasmas preincubated with pins for 2 h at 37 °C, a subsequent incubation with replenished homologous plasma was accompanied by a generation of C5a comparable to the initial incubation (data not shown). Moreover, in a single serum which was preincubated for 48 h before being replenished with fresh serum, the subsequent C5a generation was not influenced by the preincubation.

Discussion

Biodegradable polyglycolic acid or polylactide pins (Biofix®) may be useful in the treatment of several types of fractures and in stabilizing osteotomies in cancellous bone. The great initial mechanical strength combined with a strength loss during 30–50 days (PLA) and a total resorption give it an advantage compared to metal devices which frequently have to be removed after some time. However, it is highly important that biodegradable materials are biocompatible. Böstman et al. (1990) and Santavirta et al. (1990) concluded that there were merely minor nonspecific immunologic reactions of no clinical consequence when used as fracture fixation.

2 of our patients had a serious inflammatory reaction postoperatively. Other authors (Fridén and Rydholm 1992, Frøkjær and Møller 1992) have reported complications possibly caused by an unknown immunologic reaction. There have also been reports arguing against the use of PGA or PLA (Barfod and Svendsen 1992, Casteleyn et al. 1992). The present study indicates that biodegradable PLA pins may not be biologically inert. A potential of activating complement in vitro is demonstrated, which may indicate that the material is not satisfactorily compatible. We have investigated complement activation by measuring the generation of anaphylatoxin C5a des Arg. C5a, or its plasma analogue C5a des Arg, is an extremely potent chemoattractant for PMNs and macrophages and a proinflammatory mediator (Hugli 1984).

Several of the symptoms noted in our patients are thus compatible with biological effects of complement activation with release of C5a. With the high turnover of complement C5a, we did not expect the plasma control values of the patients to be different from the staff plasma controls. The main issue was to demonstrate a possible activation potential.

It is reasonable to assume that complement activation would have a larger impact when the material is in close contact with blood, plasma or interstitial fluid. Synovial fluid is thus expected to offer a favorable environment for complement activation. From the literature it appears that complications after using polylactic acid pins are more frequent when used intraarticularly (Barfod and Svendsen 1992, Fridén and Rydholm 1992).

It remains to be determined whether the material activates complement via the alternative or the classical pathway. Interestingly, also in healthy controls the complement-activating potential of polylactic acid was quantitatively not different from the patients. The finding that preincubating the material with plasma did not substantially reduce the subsequent generation of C5a des Arg deserves attention. Bioincompatible membranes used for hemodialysis are rendered more biocompatible with adsorption of plasma proteins and re-use may be a means of minimizing deleterious effects on the patient when high-degree complement activators have to be used (Jørstad 1987). The duration of the complement-activating potential of the material (PGA and PLA) during incubation in a plasma environment remains to be determined. This may also be highly relevant in vivo if the biopolymer should prove to activate complement continuously during its degradation.

The relevance of our in vitro data to in vivo conditions has to be assessed critically. The frequency of

inflammatory postoperative events, together with our demonstration of a complement-activating potential of polylactic acid pins warrant further studies on the biocompatibility of the material. Until more information is available, we have abandoned the use of polylactic acid pins for intraarticular application.

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