Mod Rheumatol (2001) 11:192–196

ORIGINAL ARTICLE

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Metal ion concentrations in the joint fluid immediately after total knee arthroplasty

Received: June 21, 2000 / Accepted: February 22, 2001

Abstract Extensive research has demonstrated increased levels of blood metal ions caused by corrosion or wear of the metal after prosthetic implantations. However, metal ion levels in the joint fluid immediately after prosthetic implantation have not been investigated. We measured the concentrations of metal ions in the joint fluid immediately after total knee arthroplasty in seven patients. Fluid specimens from the joint were obtained from the suction drain 3 days after the operation. We determined the levels of Ni^{2+}, Co^{2+}, Cr^{3+}, and Fe^{3+} using inductively coupled plasma atomic emission spectrometry (ICP–AES). Six of the seven patients exhibited high levels of Fe^{3+}, which may have been derived from the accumulated hemoglobin of the red blood cells released following postoperative hemorrhage. Ni^{2+} ions could be detected in one patient, and Cr^{3+} ions in another. These ions are probably the result of mechanical friction between the bone-saw and the cutting guide during osteotomy. We further investigated the effects of metal ions on bone-resorbing cytokine production by synoviocytes and bone marrow macrophages in vitro. The results clearly indicated that the metal ion levels detected in the joint fluid specimens were sufficient to stimulate production of these cytokines. Finally, it should be emphasized that the metal ions detected in the joint fluid in the early stages after prosthetic implantation potentially produce bone-resorbing cytokines and possibly cause subsequent periprosthetic osteolysis.

Key words Joint fluid · Metal ions · Prosthetic implant · Total knee arthroplasty

Introduction

Recently, total arthroplasty has become a popular procedure for severe joint deformities in cases of osteoarthritis (OA) and rheumatoid arthritis (RA), but complications are now being seen in greater numbers. Among these, loosening of the prosthetic implant is one of the major problems. Wear particles from the implant have been reported to be one important factor causing loosening.[1] They may accumulate locally and cause chronic inflammatory reactions which may lead to subsequent osteolysis. Some direct evidence has accumulated that phagocytosis of the wear particles by macrophages induces the release of bone-resorptive cytokines and accelerates osteolysis at the bone–implant interface.[2–7] Despite many reports in the literature regarding wear particles, there have only been a few studies on ionic metal generation. Concerns remain about the true magnitude of the release of metal ions from the implants, as well as the long-term effects of local and systemic exposure to metal ions.

Recently, several studies have been performed both in vitro and in vivo to examine the release of metal ions from Ni–Cr dental casting alloys,[8] and increased concentrations of these ions in the serum and urine have been demonstrated after prosthetic implantation.[9–11] In addition, the adverse effects of the metal ions released have been examined, especially in relation to cytotoxicity and carcinogenicity, but concentrations of the metal ions in the joint fluid immediately after prosthetic implantation and the effects of metal ions on bone-resorbing cytokine production have not been determined. In the present study, the concentrations of metal ions were measured immediately after total knee arthroplasty, and their potential for inducing bone-resorbing cytokine production was considered.

Materials and methods

Patients

Seven patients (1 male and 6 females) with successful total knee arthroplasty were studied. All the implants were of the total condylar design, and had metal components for both

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the femur and the tibia with a polyethylene insert. In three of the patients, both the femoral and tibial components were made from a Co–Cr alloy, designated American Society of Testing and Materials (ASTM) 75. In the other four, the femoral components were made from ASTM F75 and the tibial components were made from a Ti alloy, designated ASTM F136. The mean age of the patients at the time of their operation was 64.9 years (range 38–81 years). The original indication for the joint replacement was OA in two patients (two knees) and RA in five patients (five knees).

Determination of metal ions in the joint fluid

Joint fluid samples were taken from the suction drain 3 days after the total knee arthroplasty. The samples were centrifuged at 1200 g and the supernatants were stored at −80°C until analysis. After acid digestion of the samples, the concentrations of Ni^{2+}, Co^{2+}, Cr^{3+}, and Fe^{3+} were determined by inductively coupled plasma atomic emission spectrometry (ICP–AES). In two cases (patients 1 and 2), SPS 1200 (Seiko Instruments, Japan) with a detection limit of 0.05 p.p.m. was used for all the ions tested. In the other five cases (patients 3–7), SPS 4000 (Seiko Instruments) with a refined detection limit of 0.005 p.p.m. was used.

Cell preparation

Synovial membranes and bone marrow aspirates were obtained at the time of total knee arthroplasty from patients with rheumatoid arthritis satisfying the American College of Rheumatology criteria.[12] Marrow mononuclear cells were isolated by centrifugation over Ficoll Paque Plus density gradients (Pharmacia LKB Biotech., Piscataway, NJ, USA), washed twice with phosphate buffer solution (PBS), and further depleted of nonadherent cells by incubation for 16 h in plastic flasks (Becton Dickinson, Lincoln Park, NJ, USA) in DMEM (Gibco BRL, Life Technologies, Rockville, MD, USA) containing 10% FCS (Gibco BRL). The adherent cells were incubated in the presence of recombinant human M-CSF (1 ng/ml, Genzyme/Techne, USA) and GM-CSF (10 pg/ml, Genzyme, Cambridge, MA, USA) for 2 weeks to stimulate macrophagic colony formation, as described previously.[13] The synovial membranes were cut into small pieces and treated with 120 U/ml *Streptomyces* sp.C-51 collagenase (Sanko Junyaku Co., Tokyo, Japan) at 37°C for 60 min. The dispersed synovial cells were cultured in DMEM containing 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco BRL). After 24 h culture, the nonadherent cells were discarded. Synoviocytes were passaged using trypsin (Sigma Chemical Co., St. Louis, MO, USA). After this treatment, the bone marrow macrophages (above 95% purity as measured by flow cytometry) and primary-culture and 5th-passage synoviocytes (>99% $ASO2^{+}$ by flow cytometry) were used for the experiments described below.

Incubation with metal ions

$NiCl_2$, $CoCl_2$, $CrCl_3$, $Fe_2(SO_4)_3$, and lipopolysaccharide (LPS; Wako Pure Chemical Industries, Osaka, Japan) were purchased. All the metal compounds were of analytical grade and were dissolved in endotoxin-free water at concentrations of 0.01–1 mM. These concentrations had previously been found not to be cytotoxic by the MTT assay. The primary culture and 5th-passage synoviocytes and bone marrow macrophages were inoculated at a density of 3×10^5 cells/well in 48-well flat-bottomed tissue culture plates (Becton Dickinson) followed by incubation with $NiCl_2$, $CoCl_2$, $CrCl_3$ or $Fe_2(SO_4)_3$ at various concentrations. After 24 h incubation, the culture supernatants were collected and the cell lysates were extracted with lysis buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1% deoxycholate, 1% Triton X-100, 0.1% SDS, 10 mM EDTA, and 2 mM PMSF). This procedure was also carried out with LPS (1 μg/ml) as a positive control, and with medium alone as a negative control.

Enzyme-linked immunosorbent assay (ELISA)

The levels of IL-1β, IL-6, and TNF-α in the supernatants and cell lysates were measured by ELISA (Endogen, Cambridge, MA, USA). The DNA content of each well was also measured by the method of Labarca and Paigen[14] using Hoechst 33258 after papain digestion. Calf thymus DNA (Sigma) was used as a DNA standard. The data obtained from the ELISA were normalized by their respective DNA contents for quantitation.

Results

Determination of metal ions in the joint fluid

Patient 1, with ASTM F75 for the femur and F136 for the tibia, showed levels above 0.1 p.p.m. (detection limit 0.05 p.p.m.) for all the ions tested, whereas in patient 2, all the ions tested were below the detection limit (Table 1). In the other five cases, patients 3–7, the Fe^{3+} levels in the joint fluid were above the detection limit (0.005 p.p.m.). In particular, the Fe^{3+} level in patient 7 was 122.5 p.p.m. (equivalent to 2.2 mM), which may be sufficient for the induction of bone resorption. Regarding the other ions, patient 5 had an elevated level of Ni^{2+} of 0.5 p.p.m., and patient 7 had a Cr^{3+} level of 0.4 p.p.m. These levels correspond to approximately 0.01 mM and were biologically inert concentrations according to the literature.[15]

Determination of levels of metal ions which stimulate bone-resorbing cytokine production

To investigate whether the metal ions detectable in the joint fluid (Table 1) stimulate bone-resorbing cytokine production or not, bone marrow (BM) macrophages were incubated with different ions and the levels of cytokines in the

Table 1. Joint fluid metal ion concentrations just after implantation in patients undergoing total knee arthroplasty

Patient No.	Age at operation (years)	Original diagnosis	Levels of metal ions detected in joint fluid (p.p.m.)	Method	Type of alloy[a]	
					Femur	Tibia
1[b]	81	OA	Ni 0.1, Co 0.1, Cr 0.1, Fe 0.3	ICP–AES[e]	ASTM F75	ASTM F136
2[c]	53	RA	–[d]	ICP–AES	ASTM F75	ASTM F75
3[b]	67	RA	Fe 10.0	ICP–AES	ASTM F75	ASTM F136
4[b]	79	OA	Fe 20.5	ICP–AES	ASTM F75	ASTM F136
5[c]	38	RA	Ni 0.5, Fe 17.8	ICP–AES	ASTM F75	ASTM F75
6[b]	65	RA	Fe 13.5	ICP–AES	ASTM F75	ASTM F136
7[c]	71	RA	Cr 0.4, Fe 122.5	ICP–AES	ASTM F75	ASTM F75

[a] The alloys of the implants were as designated by the American Society of Testing and Materials (ASTM) and the specifications shown are: ASTM F75, cobalt 59%–60%, chromium 27%–30%, nickel <1%, molybdenum 5%–7%; ASTM F136, aluminum 5.5%–6.5%, titanium 88.5%–91%, vanadium 3.5%–4.5%
[b] Total condylar, posterior stabilizer type
[c] Total condylar, cruciate retaining type
[d] Not determined (below the detection limit)
[e] Inductively coupled plasm atomic emission spectrometry; patients 1 and 2 were analyzed by SPS 1200 (Seiko Instruments, Japan); patients 3–7 were analyzed by SPS 4000
OA, osteoarthritis; RA, rheumatoid arthritis

Table 2. Some of the metal ions which stimulate bone-resorbing cytokine production by bone marrow macrophages in a dose-dependent manner[a]

	$NiCl_2$		$CoCl_2$		$CrCl_3$		$Fe_2(SO_4)_3$	
	IL-6	IL-1β	IL-6	IL-1β	IL-6	IL-1β	IL-6	IL-1β
0.01 mM[b]	31 ± 9[c]	25 ± 8	189 ± 40	10 ± 2	211 ± 25	15 ± 6	452 ± 42	25 ± 1
0.05 mM	274 ± 38	39 ± 4	523 ± 56	35 ± 8	423 ± 98	38 ± 7	791 ± 56	91 ± 3
0.1 mM	302 ± 68	80 ± 4	664 ± 74	124 ± 6	460 ± 35	44 ± 2	464 ± 6	69 ± 2
1 mM	977 ± 89	207 ± 30	420 ± 31	18 ± 1	330 ± 17	18 ± 4	414 ± 9	7 ± 1
5 mM	622 ± 138	56 ± 1	ND[d]	ND	ND	ND	147 ± 8	2 ± 1
10 mM	269 ± 4	9 ± 1	ND	ND	ND	ND	11 ± 1	2 ± 1

[a] Cells incubated with LPS or medium alone are presented as a positive or negative control, respectively. LPS (1 μg/ml): IL-1β, 475 ± 6; IL-6, 2019 ± 214. Control (medium alone): IL-1β, 9 ± 1; IL-6, 18 ± 8
[b] Values indicate the concentration of the metal ions at which cells were incubated
[c] Cytokine levels in the culture supernatants or the cell lysates are presented. Values are expressed as the mean ± SEM of triplicate determinations, and are expressed as pg/ml μg DNA. The values refer to six independent experiments performed with cells from six different RA patients
[d] Not determined because of cytotoxic concentrations

culture supernatants were determined. Initially, the experiments were performed at a broad range of concentrations, as indicated in Table 2. Cytotoxic concentrations which might depress or abolish the responsiveness of BM macrophages were excluded by MTT assay beforehand (data not shown). The effective range was found to be 0.05–10 mM for Ni^{2+}, 0.01–1 mM for Co^{2+}, 0.01–1 mM for Cr^{3+}, and 0.01–5 mM for Fe^{3+}. It is noticeable that all the metal ions detected in the joint fluid (Ni^{2+}, Cr^{3+}, Fe^{3+}) occurred in the effective range. In addition, within these ranges, bone-resorbing cytokine production increased in a dose-dependent manner, and thereafter gradually decreased beyond the optimal concentration.

Effects of metal ions on bone-resorbing cytokine production by synoviocytes

Figure 1 shows the levels of cytokines produced following stimulation with a variety of metal ions at concentrations equivalent to those actually detected in the joint fluid. The data are expressed as percentages of the cytokine levels produced by the respective cells following LPS stimulation. The accumulation of intracellular IL-1β and secretion of IL-6 were detected following stimulation with $NiCl_2$, $CrCl_3$, and $Fe_2(SO_4)_3$ in synoviocytes as well as bone marrow macrophages. In contrast, secretion of TNF-α was only minimal. Regarding IL-6 secretion, 5th-passage synoviocytes were most striking, since $ASO2^+$ fibroblast lineage cells predominate at 5th passage and they were the major producer of IL-6, as described previously.[16]

Discussion

The implants which are most commonly used for joint replacement are made of metals with polyethylene inserts. However, it has recently been noticed that wear particles from the polyethylene inserts cause chronic inflammatory reactions and subsequent osteolysis at the bone–implant interface. Therefore, metal-on-metal bearing implants are being reexamined, particularly for total hip arthroplasty, in view of the potentially marked decrease in the wear rate.[17,18] Although metal implants have been thought to be biologically inert by themselves, they are not absolutely resistant to

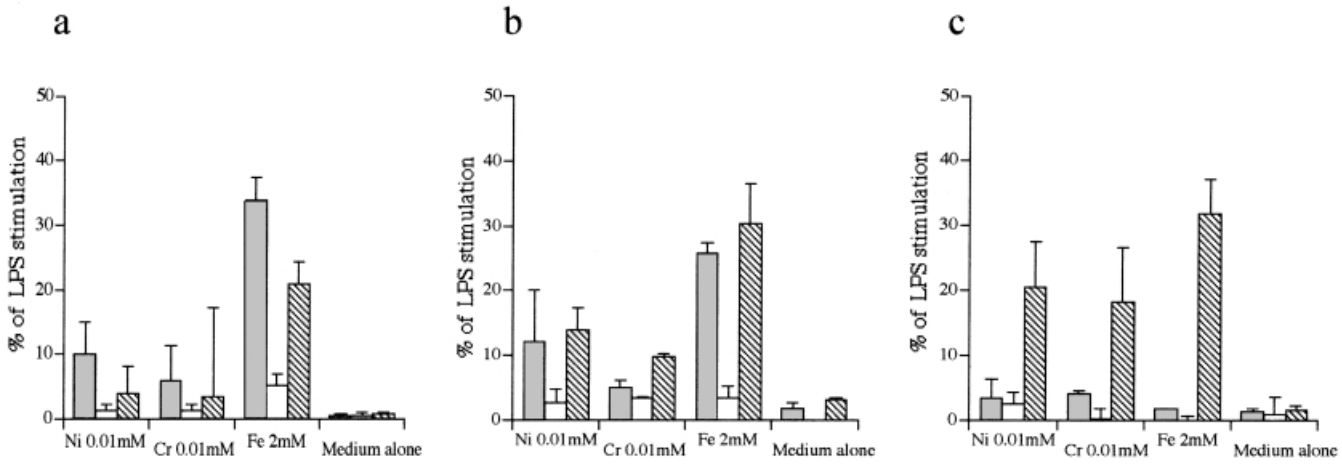

Fig. 1. Effects of metal ions on bone-resorbing cytokine production by synoviocytes and BM macrophages. BM macrophages (**a**), primary-cultured synoviocytes (**b**), and 5th-passage synoviocytes (**c**) (5×105/well) were incubated with $NiCl_2$, $CoCl_2$, $CrCl_3$, or $Fe_2(SO_4)_3$ at the concentrations indicated for 24 h. The levels of IL-1β in the cell lysates (*solid bars*), TNF-α in the culture supernatants (*open bars*), and IL-6 in the culture supernatants (*hatched bars*) are shown. The values represent the mean ± SEM of triplicate determinations, and are expressed as a percentage of the cytokine production induced by 1 μg/ml LPS (see Materials and method). The values refer to six independent experiments with cells from six different RA patients

physical and chemical influences. In fact, early osteolysis surrounding the implants has been reported within the first operative year,[19,20] and progressive loosening after metal-on-metal implantation has also been reported.[21] In the literature, increased joint pressure and soluble inflammatory mediators have been described as factors which can be responsible for periprosthetic osteolysis because of the paucity of particle generation, metallosis, and metal hypersensitivity.

In the present study, six of seven patients who underwent total knee arthroplasty showed detectable levels of Ni^{2+}, Cr^{3+}, or Fe^{3+} in the joint fluid, and these were sufficient to induce bone-resorbing cytokine production by macrophages and synoviocytes according to our in vitro data. Recent reports show that orthopaedic alloys demonstrate an extremely useful balance of corrosion and fatigue resistance, and few metal ions exist in joint fluid under physiological conditions. Therefore, the increase in metal ions in the joint fluid immediately after an operation may not be associated with the implant itself, although the source of these ions is still unclear. One possible explanation for the increased Fe^{3+} in joint fluid might be found by considering the physiological metabolism of hemoglobin following postoperative hemorrhage. In general, the red blood cells are destroyed and digested by macrophages, and released as forms of ferritin, hemoglobin, and nonprotein-bound low molecular weight iron within 24 h.[22]

Alternatively, the bone saw and the cutting guide used during the operation appear to be another source of joint fluid Fe^{3+}, since these devices are made of stainless steel which includes a high percentage of Fe (50%–60%). The increases in Ni^{2+} and Cr^{3+} may have a similar cause. It seems reasonable to suppose that the increases in Ni^{2+} and Cr^{3+} in the joint fluid can be attributed to the mechanical friction between the bone-saw and the cutting guide during osteotomy rather than to metal corrosion products derived from the implant itself. Unless the site is washed very carefully, metal ions will exist at the bone–implant interface just after the implantation. These metal ions could potentially reach high enough levels to cause periprosthetic osteolysis if they accumulate locally and are concentrated at the bone–implant interface. Therefore, they should be washed out completely during the prosthetic implantation in order to prevent metal ion-induced adverse events as well as bacterial infection.

Conclusions

We have demonstrated detectable levels of metal ions in the joint fluid in the early postoperative phase, and these were biologically active. Although periprosthetic osteolysis is multifactorial, with a variety of causes such as faulty techniques, wear particles, and stress-shielding, metal ions play a role, at least in part, in initiating bone resorption, and should be taken into account as one of important problems facing bone–implant interface biology. Further analysis of how many metal ions exist at the bone-implant interface after the implantation, and for how long, is needed for a better understanding of metal ion-induced adverse events, and this is now underway in our laboratory.

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