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Changes in Fatty Acid Profiles of Cells Surrounding Orthopedic Implants: An Experimental Study in Rats

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Abstract: Structural changes and reactions of surrounding tissues after implantation could contribute loosening in orthopaedic surgery. The aim was to investigate the effect of orthopedic implants on fatty acid (FA) profile of surrounding tissues. New Zealand rabbits (n=40) were assigned randomly to one of four commonly used orthopaedic implants (ceramic, titanium, polyethylene and stainless steel) and sham. One month after surgical intervention, the pseudomembranes developed in response to applications were harvested for FA and histopathology. The cellular FA profile was determined using gas chromatography. Fibrotic tissues developed in response to all implants. Fatty acid profile of cells surrounding implanted tissues was different from that of periosteoum (sham group). $C_{16:0}$ was the most prevalent FA in all samples. $C_{16:1}$ was absent in the stainless steel group, whereas $C_{17:1}$ was present in only in the control and ceramic groups. Fatty acid profile of the polyethylene group was similiar to that of the ceramic group. More significantly, tissues facing titanium and stainless steel contained neutophil infiltration, lympho-plasmocytes at the center of fibrotic connective tissues, macprophages and gigantic cells. As compared with ceramics and polyethylene, metallic materials may prone to greater risk for loosing due to remarkeable structural changes in surrounding tissues facing implants.

Key words: Fatty acid, orthopedic implement

INTRODUCTION

Recent studies coping with elucidation of mechanism by which factors contribute implant loosening concern periimplant tissues[1,4]. Revision surgery and postmortem cadaver studies showed generation of pseudomembrane between bone and implant, which was postulated to be a contributing factor for implant loosening[5]. Corrosions due to implant have been shown in perifer of implantation[6]. Reactions develop to both intermediate metabolites releated to this corrosion and implant material itself. This may also cause osteolysis and aseptic looseing[7].

Joint replacement and the treatment of fractures involve in the implantation of prostheses of different materials. Joint replacements may loosen aseptically (the most frequent long-term complication) and implants may break, even without welding fractures[2,8,9]. Investigations of implant failure have addressed both the implants and the patient's tissues[10]. Different implant designs and chemical composition impose different mechanical and biologic ergonomics. Various implant materials and surface covers have been tried, but implant loosening cannot always be prevented[3,11-13]. In order to broaden understanding implant failure, events occurring in the implanted bone and the surrounding tissues should be investigated at the cellular level [5,6,14]. Cells adapt in order to survive physiological stress and pathological stimuli. The ability to withstand damage depends on the type of the cell and its nutritional and hormonal status. If the adaptive capacity of the cell is overwhelmed, the cell is damaged irreversibly and dies. Cell death may follow exposure to toxins or persistent ischemia. It is difficult to exactly locate the site of the damage; however, loss of membrane integrity will cause disturbed intercellular ionic or osmotic homeostasis[15]. Cell membrane integrity is affected by the implant used. Membrane phospholipids are different outside and inside the cell, i.e. there is phospholipids asymmetry, which is maintained by the ATP-dependent enzyme phospholipids translocase. The enzyme is affected by implantation, thus membrane symmetry is lost in cells surrounding an implant, as the membrane fatty acid profile changes. Macrophages perceive the cell as an alien and proceed to phagocytes. Thus there is a need for an indicator of the changes of membrane fat acid, which would in turn indicate cell damage[9]. Fatty acid (FA) changes in biological tissue are easily detected by an automated gas chromatography system with a computer interface and soft-ware, originally developed for microbial identification, but recently also

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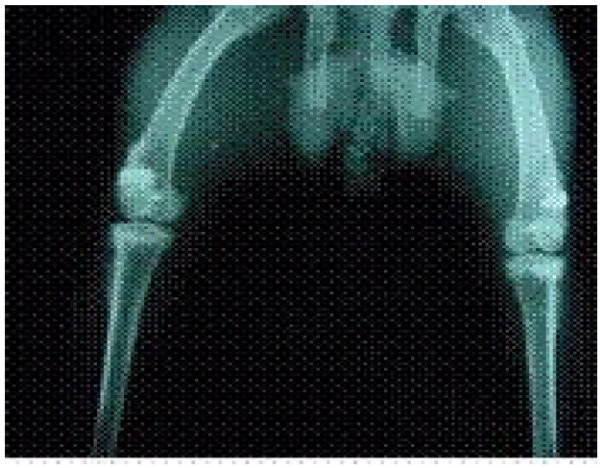

Fig.1: X-ray image of the rabbit at the one month after intervention

used for the characterization of FA profiles of higher organisms[15]. The aim of this study was to investigate the effect of orthopedic implants based on FA profiles in peri-implant tissues of New Zealand male rabbits.

MATERIALS AND METHODS

Preparation of Animals and Implant Insertion: After obtaining approval from the Medical Faculty Ethics Committee, four different implants, average 5 x 8 mm, in commercially available ceramic, titanium, polyethylene and stainless steel (Tipsan Medical Device Co. Turkey), were sterilized and prepared. Forty New Zealand Albino Rabbits, all male, aged 6 months weighing an average of 2.5 kg, kept in spacious cages, two animals per cage, with free access to feed and water, were randomly assigned to five groups of eight replications. Group 1 (ceramic), Group 2 (titanium), Group 3 (polyethylene), Group 4 (stainless steel) and group C (control). The animals were restricted to feed for one day before surgery. Xylasine hydrochloride (Rompun ® Bayer) 10 $mgkg^{-1}$ and 50- $mgkg^{-1}$ ketamine hydrochloride (Ketalar®, Eczacibasi, Turkey) were given intramuscularly. Both back legs were shaved from the ankle to the groin, prepared with polyvinylpyrolidone-iodine (polyiod ® Dragsan.Turkey) and draped with sterile towels. A 2 cm skin incision was made from the upper knee to the supracondylar femur and the implant was inserted by created implant bed. In control group defective implant bed area left empty. Cefazolin Sodium, 30 $mgkg^{-1}$, was given intramuscularly for three days post-operatively. Diclofenac sodium, 5 $mgkg^{-1}$, was administered intramuscularly on the 3rd post-operative day. X-rays were also taken to 1 month after operation (Fig. 1).

Animal Sacrifice and Specimen collection: The animals were sacrificed by thiopental overdose on day 30. The implant and surrounding tissue were carefully removed, rinsed in ice-cold physiological saline solution, weighed and then immediately subjected to fatty acid analysis.

Analysis and Extraction of Fatty acid Methyl Ester (FAME): Small pieces from each implant and control tissues were excised and used for fatty acid extraction and analysis, as described in the manual of the Microbial Identification System (Sherlock Microbial Identification system version 4.0) with the eukary database of FAME profiles for eukaryotic cells (Sherlock Microbial Identification System, version 4.0) (Sasser, 1991). In brief, approximately 30 mg of tissue from each subject was collected, then added to 1.2 $_M$ NaOH in 50% aqueous methanol with 5 glass beads (3 mm dia) in a screw cap tube and finally incubated at 100 °C for 30 min in a water bath. After the saponified samples were cooled at room temperature for 25 min, they were acidified and methylated by adding 2 mL^{-1} 54% 6 $_N$ HCl in 46% aqueous methanol, followed by incubation at 80 °C for 10 min in a water bath. After rapid cooling, methylated fatty acids were extracted with 1.25 50% Methyl-tert butyl ether (MTBE) in hexane. Each sample was mixed for 10 min and the lower phase removed with a Pasteur pipette. The top phase was washed with 3 mL^{-1} 0.3 $_M$ NaOH. After mixing for 5 min, this phase was removed for analysis. Following the base wash step, the extract (FAMEs) was cleaned in anhydrous sodium sulfate and then transferred into a GC sample vial for analysis.

FAMEs were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA, U.S.A.) with a fused-silica capillary column (25 m by 0.2 mm) with cross-linked 5% phenylmethyl silicone (HP 19091B-102). The operating parameters for the study were set and controlled automatically by a computer program. The chromatograms with peak retention times and areas were produced on the recording integrator and were electronically transferred to the computer for analysis, storage and report generation. Peak naming and column performance was achieved through the use of a calibration standard mix; Microbial ID 1201-A. Cellular fatty acids were identified on the basis of equivalent chain length data. Comparing the commercial eukary database with the MIS software package identified FAME profiles[16].

Histological Evaluation: After the sacrification, the pseudomembranous tissues were collected and fixed with 4 % buffered formaline for 48 h. The samples were dehydrated in graded series of alcohols and embedded in

Table 1. FAME[a] profiles of peri-implant and control (periosteum) of rabbits.

	Experimental groups				
Fatty acids	Stainless Control	steel	Titanium	Polyethylene	Cermaic
16:1 w7c	12±3	ND[b]	6±4[b]	15±10	6±5[b]
16:0	28±1	29±7[b]	25±1	26±0.9	26±3
17:1 iso/ante	1.8±1.3	3.5±2.2[b]	5±1.8[b]	3±2	2.6±3.4
17:1 w8c	0.7±0.2	ND[b]	ND[b]	Nd[b]	0.4±0.3[b]
17:0	0.7±0.1	0.7±0.5	ND[b]	0.5±0.4	0.7±0.4
18:0	4±1	8±5[b]	15±1[b]	6±5	9.8±8[b]
20:4 w6c	2.8±2.3	6.8±6.4[b]	12±2[b]	4±5	5±7
22:4 w6c	0.8±0.9	2.6±2.3[b]	4±0.8[b]	3.5±2.4[b]	1.8±2.1

[a]Fatty acid methyl ester

[b]$p < 0,05$ as compared to control

Fig .2: Detection of invasion of the inflammatuar cell with gigantic cells in pseudomembrane on stainless steel implant (HE x 40).

paraffin blocks. The blocks were sectioned along a plane parallel to the long axis of the implants. Sections in 3-5 µm thickness were obtained and stained in hematoxylin and eosin to determine development of fibrous reactions.

Statistical Analysis: FAME scores for the five groups were analysed using the Kruskal-Wallis test; if this showed statistical significance, then pair wise comparisons were made between groups using Mann-Whitney U-tests. The significance level was set at 0.05 for all procedures. Analyses were performed using Statistics for Windows (version 1.0; Analytical Software, Tallahassee, FL) on a personal computer.

RESULTS

Unique fatty acid profiles, composed of 17 different FAs, were observed in the periosteum tissues of control animals (Table 1). In all groups, the most prevalent fatty acid was 16:0 (palmitic acid). In implanted animals, FA profiles of the peri-implant tissues were qualitatively and quantitatively different to the controls, although not greatly dissimilar to each other. For example, the 16:1 w7c unsaturated acid (palmitoleic acid) was completely absent in the stainless steel group and 17:1 w8c was found only in the controls and ceramic groups. There was also a good correlation between polyethylene and ceramic groups. In histopathological examination, there was neutrophil infiltration and lympho-plasmocytes at the center of fibrotic connective tissues facing implants. Infiltration predominantly contained lymphocyte and polimorph nuclear leukocytes. It also contained macprphages and foreign body like-multinuclear gigantic cells. Odema was present in connective tissue as well. Structures with pale brownish pigment present cytosol of macrophages in fibrotic connective tissue could be metallic implant origin (Fig. 2).

DISCUSSION

Cell death can be initiated by the triggering two mechanisms: increased intra-cellular calcium and release of free radicals. Increased intracytoplasmic calcium activates phospholipase and protease. Both damage the cell membrane; activation of phospolipase leads to an increase in lipid destruction products. Free radicals initiate catalytic reactions and lipid peroxidation of double bonded polyunsaturated membrane fats[17].

Eitel *et al.,*[18] studied the changing role of saturated and unsaturated fatty acids in cell apoptosis and saturated fatty acids led to apoptosis in insulin producing cells, causing diabetes mellitus. Gillis *et al.,*[18]showed that lipo-oxygenase inhibitors increase arachidonic-saturated acids and cause cell death. D'Lima *et al.*,[19] investigated the cytotoxic effects of free fatty acids on B and T lymphocyte cells. However, our work is the first attempt to study the relationship between fatty acid content and cell damage in tissues surrounding orthopedic implants. We used the Microbial Identification System (MIS) to examine the fatty acid profiles of tissue surrounding implants in rabbit models.

Cell damage and apoptosis are time dependent. Apoptosis can take an hour in bone but only a few minutes in brain cells. Living tissue and culture cells may respond differently to noxious agents. Cell damage and apoptosis occur in tissues surrounding implants[20]. The loss of unsaturated FAs in the lipid bilayer as a result of lipid peroxidation decreases membrane fluidity [18]. Present results suggest that at least four unsaturated FAs (17:1 iso/ante, 18:0, 20:4 w6c, 22:4 w6c) were significantly increased and three (16:1 w7c, 17:1 w8c, 17:0) were decreased after implantation. Since ceramic and polyethylene implants had rather similar effects, our findings further expand existing knowledge. Greater changes were seen in tissues surrounding stainless steel or titanium steel implants; fatty acids were reduced, disappeared completely, or even increased compared to the control group (Table 1).

We therefore believe that ceramic and polyethylene implants cause much less reaction, i.e. cell damage, than metal implants, which are more likely to fail. Decreased membrane fluidity, leading to changes in structure and function, may play a significant role in implant failure. Thus to reduce the problem of implant failure, it is necessary to use materials which minimally disturb the surrounding tissues. Several studies have addressed this problem.

CONCLUSIONS

Fatty acid profiles can indicate cell damage in tissues surrounding implants. This paper revealed different behavior of fatty acid composition from the tissues surrounding implants, stainless steel, titanium, polyethylene and ceramic. Ceramic and polyethylene implants cause less damage than stainless steel and titanium implants, a fact, which must be taken into account when studying implant failure.

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