Acta Biomaterialia 8 (2012) 1146–1155

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Involvement of extracellular Hsp72 in wear particle-mediated osteolysis

Gema Vallés [a,b], Eduardo García-Cimbrelo [c,b], Nuria Vilaboa [a,b,*]

[a] Unidad de Investigación, Hospital Universitario La Paz-IdiPAZ, Paseo de la Castellana 261, 28046 Madrid, Spain
[b] Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain
[c] Departamento de Cirugía Ortopédica y Traumatología, Hospital Universitario La Paz, Paseo de la Castellana 261, 28046 Madrid, Spain

ARTICLE INFO

Article history:
Received 21 September 2011
Received in revised form 2 December 2011
Accepted 5 December 2011
Available online 13 December 2011

Keywords:
Osteolysis
Wear particles
Cytokines
Hsp72
Macrophages

ABSTRACT

Wear particle-mediated osteolysis is one of the major problems affecting long-term survival of orthopaedic prostheses, frequently progressing to failure of fixation and revision surgery. Upon challenging with wear particles, macrophages and various other types of cells release soluble factors that stimulate the resorptive activity of osteoclasts and impair the function and activity of osteoblasts. Extracellular Hsp72 has been reported to activate macrophages and up-regulate pro-inflammatory cytokine production, although its role in osteolysis has not been established yet. The purpose of our study was to evaluate the involvement of this protein in the inflammatory response to wear particles that leads to periprosthetic osteolysis. To this end, we used interfacial tissues and blood samples from patients undergoing revision surgery due to aseptic loosening of cementless acetabular cups. Confocal microscopy indicated that Hsp72 co-localises with $CD14^+$ cells of interfacial tissues. Levels of Hsp72 in the culture media from periprosthetic membranes cultured ex vivo decreased along culture time and Hsp72 levels in sera from patients were lower and under the assay detection limit compared with those from age-matched control subjects. This suggests that interfacial tissues are not actively producing the protein but likely recruit it from peripheral circulation. Incubation of human macrophages with titanium (Ti) particles decreased the release of Hsp72 into culture media. Treatment with recombinant human Hsp72 enhanced considerably IL-6 levels in culture media which were not modified after macrophage co-stimulation with Ti particles, while pre-incubation with Hsp72 increased the Ti particle-induced TNF-α and IL-1β production. Altogether, these data indicate that extracellular Hsp72 amplifies the inflammatory response to wear debris by interacting with resident macrophages in periprosthetic tissues.

1. Introduction

In total hip replacements, osteolysis associated to wear debris particles is one of the major problems affecting the long-term survival of prostheses, frequently progressing to failure of fixation and revision surgery. This is the end result of a cell-mediated, foreign-body inflammatory response triggered primarily by particle-activated macrophages that release several soluble factors including pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6). This situation affects the bone remodelling balance towards an osteolytic process and progresses over time to the formation of granulomatous tissue, which compromises the osseointegration of the implant [1–4]. Modification techniques developed to increase the anchorage between the bone and the implant, such as porous coatings, have remarkably decreased the long-term loosening of stems, but have not affected or have only slightly improved the results for the acetabular components [5,6]. Rates of loosening of the cup due to osteolytic lesions are progressively increasing, reaching 20% in some series 10 years after implantation [7,8]. Wear debris originating in the artificial joint space can reach the trabecular bone close to the dome of the acetabulum along the bone–material interface, through unfilled screw holes, or via non-ingrown areas of the shell. Both the periprosthetic membrane and joint fluid favour access of these particles to the acetabular area, where debris perpetuates the inflammation [4,9,10].

The stress-inducible 72-kDa form of the heat shock protein 70, Hsp72, has recently received attention due to its active role in inflammation. In addition to its chaperone and cytoprotective role during the stress response, the induction of intracellular Hsp72 in cells from the monocyte-macrophage lineage represses the expression of pro-inflammatory cytokines [11,12]. More recently, it has been documented that extracellular Hsp72 has potent immunomodulatory effects on the cellular immune response [13]. When added exogenously, Hsp72 induces cytokine production in cultures of human peripheral blood mononuclear cells, monocytes, and macrophages [14,15]. Although the exact mechanisms are not yet

* Corresponding author at: Unidad de Investigación, Hospital Universitario La Paz-IdiPAZ, Paseo de la Castellana 261, 28046 Madrid, Spain. Tel.: +34 912071034; fax: +34 917277524.
E-mail address: nvilaboa.hulp@salud.madrid.org (N. Vilaboa).

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doi:10.1016/j.actbio.2011.12.001

fully elucidated, the extracellular release of Hsp72 may result from necrotic/lytic cell death but it is also recognised that it may be secreted by active mechanisms [12,16,17]. Thus, circulating levels of Hsp72 increase after exposure to physiological stressors and in conditions in which necrotic cell death occurs, e.g. in trauma [11,16,18]. The cytokine-like behaviour of Hsp72 enables it to act in autocrine and paracrine fashions on neighbouring cells and at distant sites through its access to the bloodstream [13]. It has been suggested that the release of Hsp72 into the extracellular environment either from activated (“stressed”) or necrotic cells, may serve as an impending alarm signal or messenger in order to initiate and direct an inflammatory response. Extracellular effects or “chaperokine” activity of Hsp72 are mediated by specific receptors located in target cells to which this protein binds with high affinity and specificity. In cells of the monocyte-macrophage lineage, the signal transduction pathway initiated by Hsp72 is mainly CD14-dependent [14–16].

This study aimed to evaluate the participation of extracellular Hsp72 in the scenery provided by the presence of debris in the periprosthetic tissues, including activation of cells of the monocyte-macrophage system and release of pro-inflammatory cytokines. To this end, we assessed the expression of Hsp72 and its receptor, CD14, in periprosthetic tissues retrieved from patients undergoing revision surgery due to osteolytic lesions around the acetabular component. Next, we evaluated the release of this protein from these tissues as well as the circulating levels in the same patients. Finally, we studied the influence of extracellular Hsp72 on the secretion of pro-inflammatory cytokines typically involved in the cellular response to wear particles, employing in vitro models of macrophages and osteoblasts treated with titanium (Ti) particles.

2. Materials and methods

2.1. Tissue and blood samples

Periprosthetic interfacial tissue specimens and blood samples were obtained from 11 patients who underwent revision surgery and clinically documented for aseptic loosening. The group consisted of patients (mean age 69.6 ± 12.3 years) with a period between primary total hip arthroplasty and revision surgery of 10.9 ± 3.3 years. Details of patients are shown in Table 1. All patients had a cementless, failed modular Ti-based cup with a cobalt-based alloy against an ultra-high-molecular-weight polyethylene (UHMWPE) component in the friction couple (Table 2).

Periprosthetic membranes were used for immunofluorescence studies and tissue explant cultures. Each tissue specimen was aseptically dissected from underlying bone, fragmented, and placed in cryoblocks embedded in Tissue-Tek® Optimal Cutting Temperature Compound (OCT, Lab-Tek Products, a division of Miles Laboratories, Elkhart, IN) in random orientation to avoid possible artefacts. Samples were then snap frozen by immersion in isopentane pre-cooled at −80 °C, and stored at −80 °C. The remaining fragments were processed immediately according to the protocol described in the Confocal microscopy section. General histological analysis of the membrane specimens was performed on randomly cut sections using hematoxylin-eosin staining.

Table 1
Details of patients undergoing revision surgery due to aseptic loosening of cementless acetabular component.

CASE	AGE (years)	GENDER[a]	ETHIOLOGY (primary)[b]	TIME REVISION (years)	LOCALISATION[c]	ACETABULAR DEFECT[d]
1	67	F	OA	12.2	R	3A
2	76	F	OA	10.9	R	3B
3	57	F	OA	14.9	L	3A
4	76	M	OA	5.3	R	2A
5	38	F	SF	7.5	L	2C
6	75	F	OA	14.4	R	2A
7	80	F	OA	6.6	R	3B
8	75	F	OA	10.6	R	3B
9	77	F	OA	14.6	R	3A
10	69	F	L	11.1	L	2A
11	76	F	OA	12.1	R	2B

[a] F: female; M: male.
[b] OA: primary osteoarthritis; SF: subcapital fracture; L: luxation.
[c] R: right hip; L: left hip.
[d] Acetabular defects according to Paprosky classification [36].

Table 2
Details of loosened prostheses retrieved in revision surgery.

Case	Stem	Cup	Supplier	Friction couple[a]	HA-Coated cup[b]	Additional fixation
1	Profile HA-coated	Duraloc 500	Johnson and Johnson, DePuy, Warsaw, IN	Co-Cr-Mo/UHMWPE	No	Spikes
2	Flex Fit	Flex Fit	Traiber, Reus, Tarragona, Spain	Co-Cr-Mo/UHMWPE	No	Screws
3	Profile HA-coated	Duraloc 500	Johnson and Johnson, DePuy, Warsaw, IN	Co-Cr-Mo/UHMWPE	No	Spikes
4	Profile HA-coated	Duraloc 500	Johnson and Johnson, DePuy, Warsaw, IN	Co-Cr-Mo/UHMWPE	No	Spikes
5	Harris Galante I	Harris Galante I	Zimmer, Warsaw, IN	Co-Cr-Mo/UHMWPE	No	Screws
6	Flex Fit	Flex Fit	Traiber, Reus, Tarragona, Spain	Co-Cr-Mo/UHMWPE	No	Screws
7	Profile HA-coated	ACS Profile-ACS	Johnson and Johnson, DePuy, Warsaw, IN	Co-Cr-Mo/UHMWPE	No	Screws
8	Profile HA-coated	Duraloc 500	Johnson and Johnson, DePuy, Warsaw, IN	Co-Cr-Mo/UHMWPE	No	Spikes
9	Omnifit	Omnifit	Stryker, Allendale, NJ	Co-Cr-Mo/UHMWPE	Yes	Threaded
10	Omnifit	Omnifit	Stryker, Allendale, NJ	Co-Cr-Mo/UHMWPE	Yes	Threaded
11	Harris Galante I	Harris Galante I	Zimmer, Warsaw, IN	Co-Cr-Mo/UHMWPE	No	Screws

[a] Co-Cr-Mo: cobalt-chromium-molybdenum alloy; UHMWPE: ultra-high-molecular-weight polyethylene.
[b] HA-COATED CUP: hydroxyapatite-coated acetabular cups.