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Study of biomaterial-induced macrophage activation, cell-mediated immune response and molecular oxidative damage in patients with dermal bioimplants

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ABSTRACT

Several soft-tissue dermal fillers have been reported to provoke immunogenicity and may cause adverse reactions despite claims regarding their safety. This study aimed to assess biomaterial-induced macrophage activation, cell-mediated immune response and oxidative stress in 169 patients with dermal bioimplants. To this end, we analysed plasma concentrations of myeloperoxidase (MPO), the chitinase-like proteins chitotriosidase and YKL-40 and molecular oxidative damage. The present study shows, for the first time, that the components of innate immunity: chitotriosidase and YKL-40, are significantly higher in patients with certain bioimplants and these markers of monocyte/macrophage activation rose progressively as adverse reactions (AR) evolved. Plasma MPO levels increased 4-fold in filler users with AR and 3-fold in those without. Analysis by filler type showed subjects injected with calcium hydroxylapatite, methacrylate, acrylamides and silicone to have values significantly above those of non-filler subjects for at least two plasma biomarkers, probably because the afore-mentioned biomaterials are permanent and prone to trigger AR in the long term. By contrast, hyaluronic acid alone elicited little immune response. Plasma concentrations of markers of oxidative damage to lipids and proteins were found to be significantly higher in users of four of the nine dermal fillers studied. These diffusible products of molecular peroxidation would stem from the reaction catalysed by MPO that generates potent oxidants, leading to cell oxidative damage which, in turn, may exert deleterious effects on the organism. Overall, the results of this study on the effects of a range of dermal fillers point to chronic activation of the immune response mediated by macrophages and PMNs. The increases in plasma of MPO, chitotriosidase and YKL-40 proteins and products of macromolecular peroxidation suggests that these molecules could serve as blood-based biochemical markers and alert to the risk of chronic immune system activation and development of adverse events that may arise from the use of certain bioimplants.

Introduction

An ever-increasing number of people seek medical solutions for ageing skin or for purely aesthetic, cosmetic or medical reasons including breast cancer, congenital deformities, trauma or fat atrophy secondary to effects of highly active antiretroviral therapy. Although manufacturers and different publications claim that the fillers are non-toxic, non-immunogenic and complications are rare (Engelman et al. 2005), unwanted side effects occur with all compounds used (De Boulle 2004; Andre et al. 2005; Duffy 2005), as we and others have been able to demonstrate (Alijotas-Reig and García-Giménez 2008; Alcalay et al. 2003; Rossner et al. 2009). The many different dermal fillers currently available can be classified by product composition (silicone, collagen, hyaluronic acid (HA), poly-L-lactic acid and others) or by the length of time they remain in tissue (temporary, semipermanent or permanent) (Sánchez-

Abbreviations: AOPPs, advanced oxidation protein products; AR, adverse reactions; CAHA, calcium hydroxylapatite; ChT, human chitotriosidase; FACS, fluorescence-activated cell sorting; FLPs, fluorescent peroxidation products; HA, hyaluronic acid; HLA-class II, human leukocyte antigen-class II; HOCl, hypochlorous acid; IL-2, interleukin-2; MACR, methacrylate; MHA, methacrylate mixed with hyaluronic acid; MPO, myeloperoxidase; NASHA, non-animal stabilised hyaluronic acid; PBMCs, peripheral blood mononuclear cells; ROS, reactive oxygen species; RNS, reactive nitrogen species; PACR, polyacrylamide; PAIM, poly-alkyl-imide; PLLA, poly-L-lactic acid; PMNs, polymorphonuclear leukocytes; SMG, silicone medical grade; YKL-40, human chitinase-3-like 1.

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Carpintero et al. 2010). The most common temporary dermal fillers are autologous fat, HA, collagen (bovine, human or porcine), poly-L-lactic acid and calcium hydroxylapatite (Glasgold et al. 2007; Clark 2007; Matarasso 2007; Vleggaar and Bauer 2004; Golberg 2006), although some authors consider the latter to be semipermanent since it lasts longer than HA or collagen fillers (Ogden and Griffiths 2008). Polymethyl-methacrylate microspheres (in bovine collagen or HA) (Piacquadio et al. 2008), acrylamide and silicone are considered permanent dermal fillers (Sánchez-Carpintero et al. 2010).

The incidence of complications of temporary dermal fillers is very low (Gladstone and Cohen 2007), whereas granulomatous reactions are more frequent after treatment with permanent filler materials (Sánchez-Carpintero et al. 2010). Most late-onset adverse events associated with dermal fillers appear to be due to type IV hypersensitivity reactions since biopsies have yielded histological evidence of biomaterial-induced cell-mediated lymphomonocytic inflammatory reactions (Alijotas-Reig and García-Giménez 2008). Although T cells, macrophages and giant cells are important players in the immune response to dermal fillers, the cell line with the leading role remains elusive. The appearance of CD4-positive cells in tissue sections from punch biopsies of granulomas of patients with permanent and temporary dermal fillers has been previously reported (Lemperle et al. 2009). However, FACS analysis of peripheral blood mononuclear cells (PBMCs) did not reveal an increase in activated T cells (Alijotas-Reig et al. 2010a,b). Furthermore, neither HA nor silicone were able to elicit direct activation of unstimulated cultured PBMCs, while evaluable levels of macrophage-derived pro-inflammatory cytokines were observed in supernatants of cultured PBMCs (Alijotas-Reig and García-Giménez 2008; Miro-Mur et al. 2009). Consequently, monocyte–macrophage activation could play an important role in this kind of immune reaction.

Human chitotriosidase (ChT), a component of the innate immunity that plays a role in defence against chitin-containing pathogens, is selectively expressed and highly regulated in chronically activated lipid-laden tissue macrophages and, to a lesser degree, by PMNs (Boot et al. 1995; van Eijk et al. 2005). Plasma activity of this phagocyte-derived enzyme is markedly elevated in serum of patients with lysosomal lipid storage disorders, particularly Gaucher and Niemann-Pick diseases, but also in other pathological conditions such as, β-thalassaemia, sarcoidosis, multiple sclerosis, atherosclerosis and, particularly, in parasitic infections such as *Plasmodium falciparum* malaria (Bussink et al. 2006). In human plasma, ChT activity is currently used as a biochemical marker of macrophage activation in some lysosomal diseases. However, no data on dermal filler-related monocyte–macrophage activation, represented by the hydrolytic enzyme chitotriosidase, have been reported to date.

Another member of the chitinase-like proteins, YKL-40, also called human cartilage glycoprotein-39, is secreted *in vitro* by a variety of cells and seems to be involved in activation of the innate immune system. Although the physiological function of YKL-40 is unknown in detail, this glycoprotein has been shown to play a role in pathological conditions leading to tissue remodelling and fibrosis and appears to be pivotal in the differentiation of monocytes to activated macrophages in tissues with inflammation (Johansen 2006; Rathcke and Vestergaard 2006). To our knowledge, no data on dermal filler-related monocyte–macrophage or PMN activation have been reported to date.

Myeloperoxidase (MPO), a heme enzyme abundantly expressed by PMNs and, to a lesser extent, by monocytes and macrophages has been widely used as a marker of neutrophil activation. By its ability to generate potent chlorinated oxidants and other highly reactive species, MPO plays a major role in the antimicrobial activity of PMNs, thereby boosting physiological innate host defences (Klebanoff 2005). However, the uncontrolled or chronic formation of MPO-derived oxidants, including hypochlorous acid (HOCl), tyrosyl radical and nitrogen dioxide, which may further react with HOCl to form the highly reactive compound nitryl chloride at sites of inflammation, can contribute to progressive tissue damage in chronic inflammatory states and thus be implicated in various disease processes (Hansson et al. 2006). Furthermore, stable MPO-oxidation end-products have been shown to play a major role in the inflammatory response, thereby contributing significantly to additional cell and tissue damage (Lau and Baldus 2006).

Besides their beneficial effects against pathogens, the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by MPO of activated PMNs results in oxidative stress and nitrosative stress that can exert harmful effects on biological systems. In fact, the excess of ROS/RNS can damage cellular lipids and membranes, proteins or DNA, thereby inhibiting their normal function. As a consequence, oxidative stress has been implicated in a number of human diseases as well as in the ageing process (Valko et al. 2007).

This study aimed to assess biomaterial-induced macrophage activation, cell-mediated immune and inflammatory response in patients with dermal bio-implants, widen our understanding of the role of chitotriosidase, YKL-40 and MPO in inflammatory AR related to dermal fillers and ascertain whether these markers could foresee the development of clinical symptoms. Using immunohistopathology, skin biopsies were examined to seek possible cell-mediated inflammation associated with delayed Type IV hypersensitivity. A further aim was to study whether biomaterials induce molecular oxidative damage in patients with dermal bio-implants.

Materials and methods

Patients

Two hundred and sixty cases were selected between January 2007 and December 2008; of these, 169 were treated with dermal fillers and 91 served as age-matched non-filler user controls. Medical records and blood samples of the 260 cases (210 women, 50 men; mean age: 49 years; range: 30–65) were obtained for the study (Fig. 1).

Patients injected with dermal fillers were divided into two subgroups: one included 75 patients who suffered late-onset adverse reactions (AR) and the other comprised 94 cases with no AR related to these fillers (Fig. 1). Late-onset adverse reactions were defined as those with onset more than 6 months after bioimplant injection of one or more of the following clinical signs: oedema, angioedema, skin induration, swelling/tender nodules with or without fistula formation or discharge of sterile pus or filler material. Systemic symptoms included slight fever, arthralgia, arthritis, skin lesions, dry eyes and mouth and any other sign or clinical complaint, depending on the organ involved. No cases had either local or systemic infection when the blood samples were taken. All patients suffering AR had at least one marker of biochemical inflammatory activity when blood samples were drawn (C-reactive protein/fibrinogen, calcium, antinuclear antibodies, rheumatoid factor/Waaler-Rose, angiotensin-converting enzyme (ACE), proteinogram, complement protein C4 or lactic dehydrogenase) and 17 were under treatment with corticosteroids. Age-matched controls without filler and with an uneventful medical history served as a control group (91 cases). All participants provided informed written consent and the study was approved by the Ethics Committee of the University Hospital Vall d'Hebron.

Blood sample collection, processing and analytical methods

Peripheral blood was collected in fasting state by standard venipuncture into vacuum tubes with EDTA. Plasma was