On titanium release from dental implants and the inflammatory response

Mattias Pettersson

Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av odontologie doktorsexamen framläggs till offentligt försvar i Sal B 9 tr., byggnad 1D, målpunkt T, Fredagen den 1 juni, kl. 13:00. Avhandlingen kommer att försvaras på svenska.

Fakultetsopponent: Professor Ann Wennerberg
Institutionen för odontologi, sektion 2, Sahlgrenska akademin, Göteborgs universitet, Göteborg, Sverige.
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Abstract
In dentistry, dental implants have become a standard treatment for single tooth loss and partial and total edentulism since their introduction by P-I Brånemark in the 1960s. Long-term follow-up studies have shown that dental implantation is a predictable treatment, with an overall implant survival over ninety-five percent. Mucositis and peri-implantitis are types of inflammation in the peri-implant soft tissue, and the latter occurs with the simultaneous loss of supporting bone. The pathogenesis of mucositis and peri-implantitis is considered a microbial infection in the peri-implant tissue that causes bone loss induced by inflammation. Immune and resident cells are activated by bacterial products and toxins, which induce the release of a cascade of proinflammatory cytokines and chemokines that can activate osteoclasts and cause further bone resorption. Noninfection-induced inflammatory reactions caused by wear particles from an orthopedic implant leading to loss of the prosthesis is a well-known condition in orthopedics. This immune response induced by metal particles has been shown to act by the assembly of a protein complex, i.e., an inflammasome, in macrophages, leading to the release of proinflammatory cytokines, e.g., interleukin 1 beta (IL-1β). Whether metal particles from a dental implant are associated in the pathogenesis of peri-implantitis has not yet been investigated thoroughly. Although titanium dioxide (TiO₂) nanoparticles are known to induce a proinflammatory response, the relation between titanium (Ti) and peri-implantitis is not known.

The overall aim of this thesis was to gain knowledge of the proinflammatory capacity of Ti and its potential association with the pathogenesis of peri-implantitis. The null hypothesis in this thesis is that Ti has no proinflammatory effect.

To investigate the proinflammatory capacity of Ti, we exposed macrophages derived from a human cell line and monocytes isolated from human blood to Ti. We identified the activation and release of the proinflammatory cytokine IL-1β after the exposure of human macrophages to Ti ions, indicating activation of the inflammasome complex. A five-fold increase in the release of IL-1β was found when cells were primed with bacterial products, e.g., Escherichia coli lipopolysaccharide (E. coli LPS) prior to exposure to Ti in culture medium. The proinflammatory effect of Ti was shown to be mediated by metal-protein aggregates formed in the medium and phagocytosed by macrophages.

The exposure of macrophages to E. coli LPS mediates the production of intracellular pro-IL-1β, and a second stimulus is needed to cleave the proform of the cytokine, resulting in active IL-1β. Caspase-1, an intracellular protein, is activated through the assembly of the inflammasome complex and is needed for the activation of pro-IL-1β into its active form. Our findings indicate that the Ti-induced activation and release of IL-1β is mediated through the inflammasome complex, as the effect was reduced in the presence of a caspase-1 inhibitor. Peri-implantitis and periodontitis soft tissue samples were investigated chemically and microscopically, and a high content of Ti could be identified in the peri-implantitis tissue samples. The Ti particles identified in the peri-implantitis soft tissue might aggravate the inflammatory response and jeopardize the peri-implant treatment outcome. Transmission electron microscopy (TEM) was used to visualize the formed Ti-protein aggregates, and we discovered that the morphology of the aggregates differed in the presence of cobalt (Co). By microscopy, we could show the uptake of Ti-protein aggregates into macrophage phagolysosomes and that the location of these aggregates differed when Co was present. The origin of the Ti particles found in peri-implantitis soft tissue is unknown, but we could show that Ti is abraded from the implant during insertion into the bone. This abrasion of Ti from the implant surface into the bone is more prominent from an implant with a rough surface than with a smooth surface.

We can conclude that Ti can act as a secondary stimulus to macrophages and activate the release of active IL-1β via inflammasome complex assembly. Additionally, Ti forms metal-protein aggregates with a proinflammatory effect that can be inhibited by the presence of Co. Peri-implantitis soft tissue samples contained high concentrations of Ti and metal fragments. Lastly, Ti particles are abraded from the implant during insertion into the bone in amounts that could be proinflammatory. The proinflammatory effect induced by Ti can act in synergy with infection-induced inflammation and cause an imbalance in the host response, leading to the progression of peri-implantitis. The null hypothesis could be rejected.

Keywords
Titanium, inflammation, dental implants, peri-implantitis

Language  ISBN  ISSN  Number of pages
English  978-91-7601-859-0  0345-7532  82 + 4 papers