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Effects of NF-κB Inhibitor on Titanium Particulate-Induced Inflammation in a Murine Model

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Background. **Activation of nuclear factor kappa B (NF-κB) signaling in response to implant particulates may be critical in the pathogenesis of implant loosening after joint arthroplasty. The purpose of this study was to investigate the inhibitory effects of pyrrolidine dithiocarbamate (PDTC) in a murine model of inflammation induced by titanium (Ti) particulates.**

Materials and Methods. **Ti particulates were introduced into established air pouches on C57BL/6J mice. Mice were injected intraperitoneally with either high-dose PDTC (100 mg/kg) or low-dose PDTC (50 mg/kg). Mice without drug treatment, as well as mice injected with saline alone were included. Each group consisted of sixteen mice. The membranes and lavage fluid were harvested 2 d or 7 d after injection of particulate suspension for histological and molecular analysis.**

Results. **Histologic analysis showed that PDTC reduced inflammatory responses in air pouches, that is, thinner membrane and decreased cellular infiltration. In addition, PDTC reduced the release of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) in the lavage fluid or supernatant of homogenates as evaluated by ELISA.**

Conclusion. **These results suggest that PDTC inhibits Ti particulate-induced inflammatory responses in the murine model; thus it represents a promising therapeutic candidate for the prevention and treatment of implant loosening.**

Key Words: **pyrrolidine dithiocarbamate; NF-κB; inflammatory response; titanium particulates; implant.**

INTRODUCTION

Total joint arthroplasty is an effective treatment for patients with severe joint diseases. However, implant loosening is the leading complication that limits survivorship of total joint arthroplasty. Despite the prevalence of this complication, few nonoperative treatments for periprosthetic osteolysis are available. The inflammatory response to wear particulates, which are primarily generated from the prosthetic implant, is widely accepted as the underlying cause of periprosthetic osteolysis and consequent aseptic loosening [1, 2]. Significantly large quantities of pro-inflammatory cytokines that stimulate osteoclast differentiation and activation are involved in the biologic response to wear particulates. In addition to their direct actions on osteoclast precursors, proinflammatory cytokines may indirectly promote osteoclast activity, through expression of receptor activator of NF-κB ligand (RANKL), the key cytokine regulator of osteoclast generation and activation [3, 4].

Several transcription factors have important roles in inflammation and osteoclastogenesis. According to recent findings, the most notable of these transcription factors in wear particulates-induced inflammation and osteoclastogenesis is NF-κB [5–7]. As part of the inflammation process, cytokines, chemokines, growth factors, and cell adhesion molecules are released and frequently overexpressed. Of these key cytokines, TNF-α and IL-1β play a critical role in the development of inflammatory bone resorption. It is the NF-κB signaling pathway that regulates the synthesis and action of inflammatory cytokines, which include TNF-α and IL-1, in the inflammatory reaction. In turn, the NF-κB pathway is activated after stimulation of these cytokines [8, 9].

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Previous studies have demonstrated the utility of pharmacologic NF-κB suppression in the inhibition of inflammatory bone destruction such as in rheumatoid arthritis [10, 11]. In numerous *in vivo* studies on inflammation, pyrrolidine dithiocarbamate (PDTC) is a well-characterized NF-κB inhibitor with demonstrated efficacy and good tolerability [12]. This inhibitor acts by stabilizing cytosolic IκB-α, thus preventing the activation and nuclear translocation of the NF-κB transcription factor. However, the use of this potent NF-κB inhibitor to blunt the inflammation associated with wear particulates has not been previously reported.

Our hypothesis for the presented study is based on a sequence of events, including aggregation and activation of macrophages during periprosthetic loosening, and activation of NF-κB after particulate stimulation, leading to inflammatory cytokine release. In the present study, we examined the effect of PDTC as an effective approach for ameliorating titanium (Ti) particulate-induced inflammation *in vivo*.

MATERIALS AND METHODS

Preparation of Particles

Pure titanium particulates were obtained from a commercial source (Johnson Matthey Chemicals, Ward Hill, MA). Using Coulter ZM Channelizer, (Coulter Electronics, Hialeah, United States) the size and distribution of particulates were valuated. Ninety percent of the particulates were <5 μm in diameter. The particulates were subsequently rinsed in 70% ethanol three times and sterilized in 70% ethanol for 48 h. Next, they were washed three times in sterile phosphate buffered saline (PBS) and resuspended in sterile PBS at 1.0×10^8 particulates/mL. The limulus amebocyte lysate was used to establish that the sterilized particle suspension was free of endotoxin.

Murine Air Pouch Model and PDTC Treatment

Mice of the C57BL/6J strain (8 wk old, female) weighing 20–25 g were obtained from the Shanghai Laboratory Animal Center (Chinese Academy of Sciences).These mice were maintained in specific, pathogen-free conditions at our animal breeding facility. All animal studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the United States National Institute of Health (National Academy Press, 1996), and approved by the Laboratory Animal Care and Use Committee of Shanghai Jiaotong University in China. In Brief, air pouches were generated on mice as described [2, 13]. Mice were divided into a negative control group (normal PBS group), positive control group (PDTC-untreated group), PDTC-treated group I (low-dose group), and PDTC-treated group II (high-dose group). After air pouch formation, pouches were injected with 0.5 mL PBS containing titanium particulates in the absence (untreated) or presence of PDTC (Sigma Chemical Co., St. Louis, MO) administration (PDTC-treated). The pouches injected with PBS alone were included as negative control. For the PDTC-treated mice, PDTC was injected intraperitoneally (100 mg/kg or 50 mg/kg) 1 d prior to injection of particulate suspension, with daily injection maintained until the mice were sacrificed. The sacrifice was accomplished through placement of the mice in a carbon dioxide chamber at 2 d or 7 d following injection of particulate suspension. Later, the pouch membranes and lavage fluid were harvested for morphological or molecular analysis.

Thickness and Cellularity of Air Pouch Membrane

Formalin-fixed pouches were embedded in paraffin and then sectioned at 6 μm with consistent orientation. These sections were stained with hematoxylin and eosin (H and E) and examined under light microscope (Olympus DP70, Tokyo, Japan) to observe pouch membrane inflammation. Simultaneously, four separate sections per specimen were analyzed using the Image-Pro Plus software package (Media Cybernetics, Bethesda, MD). Pouch membrane thickness was determined at six points on each section, with an even distribution of measurement on the proximal side, distal side, and transition curve of the pouch. The total number of cells (based upon nucleus count) was determined as cells/mm^2.

Enzyme-Linked Immunosorbent Assay (ELISA) for TNF-α, IL-1β Detection

With the use of a commercial ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions, the levels of TNF-α and IL-1β protein in pouches were detected at 2 or 7 d after injection of particulate suspension.

Statistical Analysis

Mice were randomly assigned to four experimental groups with 16 mice in each group. Statistical analysis among groups was performed by the analysis of variance (one-way ANOVA) with S-N-K *post-hoc* *t*-test. Data were shown as mean ± SD. The difference was considered statistically significant at $P < 0.05$. All statistical analyses were conducted using SPSS 12.0 (SPSS, Chicago, IL).

RESULTS

Effect of PDTC on Particulate-Induced Inflammation

The data indicate that PDTC treatment resulted in less cellular infiltration and membrane proliferation in air pouch tissue. Figure 1 illustrates how pouches injected with Ti particulates (positive control group) developed pronounced inflammatory changes (Fig. 1B) compared with pouches injected with PBS alone (Fig. 1A). PDTC treatment significantly ameliorated the inflammation within the pouch (Fig. 1C and D). Histological evaluation also revealed significant elevation of membrane thickness and number of cells per mm^2 in pouches stimulated by Ti particulates compared with the negative controls at day 2 and 7 (Fig. 2A and B, $P < 0.001$). In contrast to the positive control group, both PDTC-treated group I (Fig. 2A, $P < 0.01$) and PDTC-treated group II (Fig. 2A, $P < 0.001$) exhibited marked reduction of membrane thickness compared with the positive controls at day 2 and 7. Likewise, significant suppression of cellular infiltration was noted in PDTC-treated group I and PDTC-treated group II compared with the positive control group at day 2 (Fig. 2B, $P < 0.01$), though suppression was strongest in PDTC-treated group II at day 7 ($P < 0.001$).