The expression of sonic hedgehog in diabetic wounds following treatment with poly(methacrylic acid-co-methyl methacrylate) beads

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ARTICLE INFO

Article history:
Received 19 March 2012
Accepted 1 April 2012
Available online 26 April 2012

Keywords:
Wound healing
Angiogenesis
Diabetes
Gene expression
Sonic hedgehog
Growth factors

ABSTRACT

The expression of native sonic hedgehog (Shh) was significantly increased in poly(methacrylic acid-co-methyl methacrylate) bead (MAA) treated wounds at day 4 compared to both poly(methyl methacrylate) bead (PMMA) treated and untreated wounds in diabetic db/db mice. MAA beads also increased the expression of the Shh transcription factor Gli3 at day 4. Previously, topical application of MAA beads (45 mol% methacrylic acid) improved wound closure and blood vessel density in excisional wounds in these mice, while PMMA beads did not. Gene expression within the granulation tissue of healing wounds was studied to provide insight into the mechanism of vessel formation and wound healing in the presence of MAA beads. In addition to the increased expression of Shh, MAA-treated wounds had increased expression of osteopontin (OPN), IL-1β and TNF-α, (at day 7) similar to the previously reported MAA response of macrophage-like and endothelial cells in vitro.

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1. Introduction

The formation of new blood vessels (angiogenesis) is an essential process for tissue regeneration and tissue engineering. In wound healing, impaired angiogenesis is associated with the delayed healing of chronic wounds, including diabetic wounds [1,2]. Poly(methacrylic acid-co-methyl methacrylate) (MAA) beads have beneficial effects in skin graft and diabetic wound healing models by promoting neovascularization of the surrounding tissue [3,4]. In vitro, macrophage-like cells (dTHP-1) increased the expression of selected cytokines, while having little to no effect on the expression of pro-angiogenic growth factors [5], suggesting that MAA beads exert their effect, in part, by modulating the inflammatory response in macrophages.

In order to better understand the effect of MAA beads in vivo, and connect the in vitro response to the angiogenic effect MAA beads have in wound healing models, the expression of seventeen genes within the granulation tissue of diabetic wounds treated with MAA beads was analyzed, including genes involved in wound healing [interleukin(IL)-1β, IL-6, tumor necrosis factor alpha (TNF-α)], and osteopontin (OPN) and the regulation of angiogenesis [vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor B (PDGF-B), thrombospondin 1 (TSP-1), Sprotu 2 (Spry2) and C-X-C motif chemokine 10 (CXCL10)]. The expression of sonic hedgehog (Shh) and its pathway proteins [transmembrane receptors Patched (Ptc)1, Ptc2, and Smoothened (Smo)], and transcription factors Gli1, Gli2 and Gli3 were also analyzed as preliminary microarray data implicated Shh involvement in the host response to MAA beads (unpublished data). While best known as a developmental morphogen, Shh also contributes to post-natal angiogenesis [6] and upon delivery or overexpression (via gene therapy) has been shown to accelerate wound healing in diabetic mice [7,8]. The present study suggests that MAA beads modulate the inflammatory response and the Shh pathway, resulting in increased wound vascularization.

2. Materials and methods

2.1. Bead preparation

MAA beads (150–250 μm in diameter, 45 mol% methacrylic acid) were obtained from Rimon Therapeutics (Toronto, Canada) and PMMA control beads (same diameter, 100 mol% methyl methacrylate) were acquired from Polysciences (War- rington, PA, USA). MAA and PMMA bead preparation [5] and characterization [3,4] are described elsewhere. Briefly, beads were washed in a sonicating water bath for 20 min in 95% ethanol (MAA beads) or 1 N HCl (PMMA beads) ten times, then