ORIGINAL ARTICLES

Opioids and opioid receptors orchestrate wound repair

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We have previously shown that topical opioids including morphine and its congeners promote healing of full thickness ischemic wounds in rats. We examined the contribution of mu opioid receptor (MOPr)–mediated healing of full thickness ischemic wounds using MOPr and delta or kappa opioid receptor knockout (KO) mice. Wound closure in the early (day 5) as well as later phases was delayed in topical morphine or PBS-treated MOPr-KO mice compared with reciprocal treatments of wounds in wild-type (WT) mice. MOPr expression was significantly upregulated at 30 min in the wound margins and colocalized with wound margins and vasculature in the epidermal and dermal layers of the skin. We next examined whether neuropeptide expression was involved in the mechanism of MOPr-mediated wound closure. Substance P (SP) and calcitonin gene-related peptide immunoreactivity (ir) was significantly increased in the skin of MOPr-KO mice as compared with WT mice. Neuropeptide-ir was increased significantly in PBS-treated wounds of MOPr and WT mice, but morphine treatment reduced neuropeptide immunoreactivity in both as compared with PBS. Wounding of keratinocytes led to the release of opioid peptide beta-endorphin (β-END) in conditioned medium, which stimulated the proliferation of endothelial cells. MOPr-selective (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2, CTOP) and nonselective OPr antagonist naloxone-inhibited endothelial proliferation induced by wounded keratinocyte-conditioned medium. In addition, accelerated wound area closure in vitro by morphine was suppressed by methylnaltrexone, a nonselective OPr antagonist with high affinity for MOPr. Morphine and its congeners stimulated the proliferation of endothelial cells from WT mice but not those from MOPr-KO mice. Furthermore, morphine-induced mitogen-activated protein kinase/extracellular signal-regulated kinase phosphorylation in endothelial cells was significantly decreased in MOPr-KO mice as compared with WT mice. Collectively, these data suggest that MOPr plays a critical role in the proliferation phase with the formation of granulation tissue during wound healing. (Translational Research 2017;185:13–23)

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Abbreviations: β-END = beta-endorphin; BOECs = blood outgrowth endothelial cells; CGRP = calcitonin gene-related peptide; CTOP = D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2; DALDA = (D-Arg2,Lys4)dermorphin-(1,4)-amide; DOPr = delta opioid receptor; DMEM = Dulbecco's Modified Eagle Medium; DSS = dextran sodium sulfate; DRG = dorsal root ganglion; EGFR = epidermal growth factor receptor; FBS = fetal bovine serum; FITC = fluorescein isothiocyanate; HDMECs = human dermal microvascular endothelial cells; HUVECs = human umbilical vein endothelial cells; KOPr = kappa opioid receptor; KO = knockout; LSCM = laser scanning confocal microscopy; MAPK/ERK = mitogen-activated protein kinase/extracellular signal regulated kinase; MNTX = methylnaltrexone; MOPr = mu opioid receptor; OPrs = opioid receptors; PDGF = platelet-derived growth factor; PDGFR-β = platelet-derived growth factor receptor-β; PE = phycoerythrin; SCD = sickle cell disease; SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis; SP = substance P; VEGFR2 = vascular endothelial growth receptor 2; WT = wild-type

AT A GLANCE COMMENTARY

Wang Y, et al.

Background

Nonhealing, painful wounds in conditions including sickle cell disease and diabetes are difficult to treat. Opioids and opioid receptors are expressed in the skin and are integral to normal skin homeostasis and repair. Topical opioids such as morphine and its congeners have been shown to promote ischemic wound healing in rats.

Translational Significance

We show that the mu opioid receptor in mice mediates wound healing in the skin microenvironment by inhibiting neuropeptide expression and promoting endothelial proliferation through the MAPK/ERK pathway. These studies suggest that opioids offer a unique advantage in treating wounds because of their ability to concurrently induce healing and ameliorate pain via peripheral mu opioid receptors.

INTRODUCTION

Wound healing is an orderly and timely process resulting in the formation of new skin that is similar in strength and integrity to normal skin. However, under several pathophysiological conditions such as diabetes and sickle cell disease, the healing process is impaired, leading to chronic nonhealing wounds, often associated with pain.[1] Therefore, therapies that target the multicellular processes to promote wound healing and alleviate pain are required.

Experimental and clinical studies suggest that opioids and opioid receptors (OPrs) expressed in the skin are integral to normal skin homeostasis and repair.[2-4] Opioids and OPrs are expressed in a wide variety of tissues including the central nervous system, vasculature, tumors, skin keratinocytes, and immune cells. Opioid primarily interact with three classical OPrs: mu, delta, and kappa (MOPr, DOPr, and KOPr, respectively). These G-protein–coupled receptors bind to both endogenous opioid ligands including endorphins, enkephalins, and dynorphins and exogenous ligands such as morphine and its congeners. Best known for its antinociceptive activity, the opioid signaling axis is also implicated in wound healing.[2,3,5,6] Opioids offer a unique advantage in treating pathophysiological wounds because of their ability to ameliorate pain via peripheral OPrs, since pain is often a serious manifestation of nonhealing chronic wounds.[3,5]

Cutaneous wound healing cascade involves several phases involving hemostasis, inflammation, proliferation, and remodeling.[7] Soluble factors including growth factors and cytokines are released during the earlier phases from platelets and inflammatory cells to initiate recruitment and proliferation of cells required for the formation of new tissue. Opioids are also released from the inflammatory cells at the site of injury, while, opioid receptors are known to be activated in the periphery and dorsal root ganglion neurons in painful conditions.[3] In addition, neuropeptides released from the nerve terminals on injury have been shown to stimulate neurogenic inflammation, angiogenesis, and wound healing.[8] Release of neuropeptides during the proliferation phase may have a beneficial effect on wound healing but their sustained increase may lead to amplified inflammation and contribute to impaired healing.

Atrophy of the epidermis in MOPr and DOPr knockout mice suggests a controlling role for OPrs on keratinocyte proliferation and differentiation.[9] Angiogenesis and cell proliferation with the formation of granulation tissue are fundamental contributors to the wound healing process.[10] Morphine and other MOPr agonists stimulate angiogenesis in tumors and wounds and accelerate wound closure in normal and diabetic rats.[5,6] Therefore, we examined the contribution of MOPr on the early phase of proliferation involving endothelial proliferation and revascularization in chronic wounds.