Hyperbaric Oxygen Attenuates Apoptosis and Decreases Inflammation in an Ischemic Wound Model

Qixu Zhang[1], Qing Chang[1], Robert A. Cox[2], Xuemei Gong[1] and Lisa J. Gould[1]

The molecular mechanisms whereby hyperbaric oxygen (HBO) improves ischemic wound healing remain elusive. In this study, a rat model of wound ischemia was used to test the hypothesis that HBO enhances wound healing by modulating hypoxia-inducible factor-1α (HIF-1α) signaling. Male Sprague–Dawley rats underwent creation of a previously validated ischemic flap. Three groups underwent daily treatment: HBO (90 minutes, 2.4 atm); systemic administration of the free radical scavenger, *N*-acetylcysteine (NAC 150 mg kg^{-1} intraperitoneal); control (neither HBO nor NAC). HBO treatment improved healing of the ischemic wounds. Analysis of ischemic wound tissue extracts demonstrated significantly reduced expression of HIF-1α, p53, and BNip3. Additionally, HBO increased expression of Bcl-2 while decreasing cleaved caspase-3. DNA fragmentation was abolished and the number of TUNEL-positive cells was reduced compared to the other groups. Vascular endothelial growth factor, cyclooxygenase-2, and neutrophil infiltration were reduced in ischemic wounds treated with HBO. These results indicate that HBO improves ischemic wound healing by downregulation of HIF-1α and subsequent target gene expression with attenuation of cell apoptosis and reduction of inflammation.

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INTRODUCTION

Chronic wounds are a common but underrecognized problem that significantly impacts patients' quality of life. The wide range of therapeutic options for these wounds indicates that the mechanisms responsible for non-healing wounds are poorly understood. Hyperbaric oxygen therapy (HBOT) has been used to treat chronic wounds for about 40 years on the assumption that delivery of increased oxygen to the wound will improve healing. Several randomized, controlled trials have shown the benefit of HBOT in diabetic foot ulcer outcome as measured by reduction in the risk of major amputation (Doctor *et al.*, 1992; Faglia *et al.*, 1996; Kalani *et al.*, 2002; Abidia *et al.*, 2003; Kessler *et al.*, 2003), and yet the effect on actual ulcer healing remains elusive. Treatment of chronic wounds other than diabetic foot wounds with HBOT has not withstood the rigor of randomized controlled trials, perhaps because the heterogeneity of wounds other than diabetic foot ulcers makes such trials extremely difficult to perform (Kranke *et al.*, 2004). Thus, the utility of HBOT for healing chronic wounds remains a subject of debate. On the other hand, HBOT has been shown to protect the central nervous system from ischemia or ischemia–reperfusion injury (Baidin *et al.*, 1997; Rosenthal *et al.*, 2003). Cited mechanisms for HBO-induced neuroprotection include increased oxygen supply (Sunami *et al.*, 2000), improved cerebral metabolism (Ginsberg, 2003), reduced inflammation (Thom, 1993), attenuation of apoptosis (Calvert *et al.*, 2003; Yin *et al.*, 2003), and ischemic tolerance or ischemic preconditioning (Xiong *et al.*, 2000; Dong *et al.*, 2002). These beneficial effects suggest that oxygen has multiple roles when delivered in high concentrations to an ischemic environment. It is our premise that improved understanding of the effect of oxygen on ischemic wounds will lead to more effective, specifically targeted wound treatments.

Hypoxia-inducible factor-1 (HIF-1) plays a central role in oxygen homeostasis through a redox-dependent mechanism. Numerous studies have shown that HIF-1α and its target genes play an important role in cardiac myocyte death and brain death caused by hypoxia (Regula *et al.*, 2002; Yussman *et al.*, 2002; Graham *et al.*, 2004; Li *et al.*, 2005; Ostrowski *et al.*, 2005; Galvez *et al.*, 2006), and yet the effect of extreme hyperoxia, which also increases free radicals, is unclear. We proposed that HBO would regulate HIF-1 through increased free radical oxygen species and that regulation of HIF-1α-associated genes would play an important role in the ischemic wound healing process. In this study, we examine the effect of HBO and the free radical scavenger *N*-acetylcysteine

[1]*Division of Plastic Surgery, Department of Surgery, University of Texas Medical Branch, Galveston, Texas, USA and* [2]*Department of Pathology, University of Texas Medical Branch, Galveston, Texas, USA*

Correspondence: Dr Lisa J. Gould, Department of Surgery, University of South Florida, James A. Haley Veterans Hospital, Tampa, Florida 33612, USA. E-mail: lisa.gould@va.gov

Abbreviations: COX-2, cyclooxygenase-2; HBO, hyperbaric oxygen; HBOT, hyperbaric oxygen therapy; HIF-1α, hypoxia-inducible factor-1α; NAC, N-acetylcysteine; Pscο$_2$, subcutaneous tissue oxygen tension; TBST, Tris-buffered saline with Tween-20; VEGF, vascular endothelial growth factor

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