Does hyperbaric oxygen (HBO₂) cause gene mutations that are responsible for the late-phase antinociceptive response in mice?

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ABSTRACT. Hyperbaric oxygen (HBO₂) is approved by the FDA for a limited number of clinical indications albeit not for chronic pain. Research from our laboratory has demonstrated that HBO₂ treatment produces an antinociceptive effect of unusually long duration that involves nitric oxide (NO) and opioid mechanisms [Zylstra et al., Exptl Biol Meeting Abst, abstracts 711.16 and 711.17, 2008]. The objective of the present research is to determine whether the long-lasting effect might result due to changes in genes that regulate NO and opioid mechanisms.

INTRODUCTION. We recently reported that four daily 60-min HBO₂ treatments produce an antinociceptive effect that persists for up to three weeks. There was an early-phase response of 6 hr duration that subsided by 12 hr after HBO₂ treatment; and a late-phase response that emerged at 24 hr and lasted up to three weeks after the last HBO₂ treatment. Continuous treatment with naltrexone (NTX, an opioid antagonist) or L-N⁶-nitro arginine methyl ester (L-NAME, a nitric oxide synthase inhibitor) during the fours days of HBO₂ treatment significantly inhibited the late-phase antinociceptive effect. (See figure below.)

The purpose of the present study is to employ microarray analysis to test the hypothesis that four-day HBO₂ treatment initiates a sequence or cascade of gene changes—possibly in the NO-opioid pathway—that may be responsible for the late-phase antinociceptive response.

MATERIALS AND METHODS (Continued)
Abdominal Constriction Test. Mice will be treated i.p. with 0.6% glacial acetic acid (0.1 ml/10 g volume of injection). Exactly 5 min later, the number of abdominal constrictions—lengthwise stretches of the torso with concave arching of the back—in each animal will be counted for a 6-min period. The degree of antinociception (inhibition of abdominal constrictions) produced in various treatment groups of mice will be calculated as follows:

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\frac{\text{antinociception}}{\text{control mice}} = \frac{\text{# constrictions in control mice}}{\text{# constrictions in exposed mice}} \times 100
\]

Example of an abdominal constriction

Exposure to HBO₂, HBA, HBO₂. Different groups of animals will be placed in a B-11 research hyperbaric chamber (Reimers Systems, Inc., Lorton, VA), and subjected to four daily 60-min exposures to normobaric oxygen (HBO₂, 100% oxygen at 1 absolute atmosphere (ATA)), hyperbaric air (HBA, compressed air at 3.5 ATA) or hyperbaric oxygen (HBO₂, 100% oxygen at 3.5 ATA). Animals will be assessed for antinociceptive responsiveness using the acetic acid abdominal constriction test at 9 hr, 24 hr (1 day), and 3 days (3D) following the last HBO₂ treatment. A reference group was exposed to normobaric air (NBA, compressed air at 1.0 ATA) for 60 min.

Induction of early-phase (left) and late-phase (right) antinociceptive responses by repeated HBO₂ treatment

DISCUSSION Blockade of opioid receptors or inhibition of NO production in the brain prevents development of the late-phase antinociception. This finding implicates opioid and NO mechanisms in the long duration of antinociception. The microarray analysis will reveal whether the four-day HBO₂ treatment causes changes in genes in opioid and NO function that result in the late-phase antinociception. It is also possible that manipulation of opioid and NO during HBO₂ treatment may cause changes in genes that regulate other systems (e.g., other pathways that mediate pain relief). A better understanding of the mechanism of HBO₂-induced antinociception and identification of the factors that determine its duration of action might portend the clinical application of HBO₂ for long-term treatment of chronic pain conditions.

REFERENCES
Zylstra CC, Ohgami Y, Chung E, Shirachi DY, Quock RM. 2008 Exptl Biol Meeting Abst [on CD-ROM], abstract 711.16 (2008a)

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MATERIALS AND METHODS
Animals. Male 10 to 11-week-old NIH Swiss mice, 18-22 g body weight, will be purchased from Harlan Laboratories (Indianapolis, Indiana) and will be used in this IACUC-approved research.