Prostaglandins, Leukotrienes and Essential Fatty Acids 127 (2017) 16–19

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Hyperbaric oxygen therapy is not associated with oxidative stress assessed using plasma F_2-isoprostanes and isofurans

Tomas Corcoran[a], Sonya Ting[a], Emilie Mas[b], Michael Phillips[c], Edmond O'Loughlin[d], Anne Barden[b,*,1], Trevor A. Mori[b,a]

[a] Department of Anaesthesia & Pain Medicine, Royal Perth Hospital, Perth, Western Australia, Australia
[b] School of Medicine & Pharmacology, University of Western Australia, Australia
[c] UWA Centre for Medical Research, University of Western Australia, Perth, Australia
[d] Department of Anaesthesia, Fiona Stanley Hospital, Murdoch, Western Australia, Australia

ARTICLE INFO

Keywords:
Lipid peroxidation
Human
Hyperbaric oxygen therapy
F_2-isoprostanes
Isofurans

ABSTRACT

Background and aims: Hyperbaric oxygen (HBO) therapy is increasingly used in medical practice as a means of enhancing the formation of collagen matrix and angiogenesis, thus promoting healing in wounds and necrotic tissue. However, there are concerns that oxygen can also associate with increased production of oxygen free radicals and oxidative stress. F_2-Isoprostanes (F_2-IsoPs) formed by non-enzymatic oxidation of arachidonic acid (AA) are reliable measures for assessing oxidative stress *in vivo*. In addition, under conditions of high oxygen tension isofurans (IsoFs) are preferentially formed from AA and are considered to better reflect oxidative stress in the setting of high oxygen tension. This study aimed to measure plasma IsoFs and F_2-IsoP in patients receiving HBO therapy to treat osteonecrosis secondary to radiation therapy. Our hypothesis was that IsoFs would continue to rise with increasing oxygen pressures in contrast to F_2-IsoPs whose synthesis would be reduced.

Methods: Twelve patients receiving hyperbaric therapy to treat osteonecrosis secondary to radiation therapy were studied during hyperbaric treatment. Blood samples were collected prior to, during and after cessation of HBO therapy that lasted for 119 min. Seven serial blood samples were collected for measurement of plasma F_2-IsoPs and IsoFs, blood gases and haemoglobin.

Results: Oxygen saturation and venous oxygen partial pressure (PvO_2) rose significantly during hyperbaric therapy. However, there were no significant changes in plasma IsoFs or F_2-IsoPs during the hyperbaric therapy session.

Conclusion: In this study of patients with osteonecrosis, HBO therapy at a maximum pressure of 2.4 atm with up to 100% oxygen did not worsen oxidative stress assessed using plasma F_2- IsoFs and IsoPs.

1. Introduction

Oxygen therapy is utilised on a daily basis in modern medicine and is considered to be essential in the management of many common diseases. However, the use of oxygen, particularly at supraphysiological pressures, has the potential to increase systemic oxidative stress, or overproduction of oxygen free radicals. An imbalance of free radical production has been known to result in deleterious modification of biological macromolecules [1]. Furthermore, research over the last 50 years has implicated free radical production in the pathogenesis of an increasing number of illnesses, including hyperoxic lung disease, atherosclerosis, early retinopathy, Parkinson's disease and certain forms of cancer [1,2]. Therefore a sensitive and specific marker would be most useful to assess of the role of oxygen toxicity in the pathogenesis of these and other conditions.

F_2-Isoprostanes (F_2-IsoP) are prostaglandin-F_2 like compounds formed non-enzymatically from arachidonic acid [3]. They are considered to be one of the most reliable measures for assessing oxidative stress *in vivo* [2]. Studies of the biochemistry of the formation of F_2-IsoPs under various *in* vitro and *in* vivo conditions, showed that F_2-IsoPs became increasingly limited with elevated oxygen concentrations [3]. Under conditions of high oxygen tension a different class of compounds known as isofurans (IsoFs), also formed from arachidonic acid, are favoured [2]. IsoFs have also been shown to be elevated in the substantia nigra of patients with Parkinson's disease and hyperoxic lung injury [3]. These findings suggests IsoFs may hold exciting potential as a uniquely

* Correspondence to: University of Western Australia, School of Medicine and, Royal Perth Hospital, Box X2213 GPO Perth 6847, Western Australia, Australia.
E-mail address: anne.barden@uwa.edu.au (A. Barden).
[1] Equal senior authorship.

http://dx.doi.org/10.1016/j.plefa.2017.10.003
Received 2 May 2017; Received in revised form 28 September 2017; Accepted 6 October 2017

sensitive indicator of oxidative stress in various disease states.

To date there are no data examining the formation of IsoFs or IsoPs under conditions where patients are breathing oxygen under increased atmospheric pressure. Hyperbaric oxygen therapy (HBO) is increasingly used in a number of areas of medical practice, including carbon monoxide poisoning [4], decompression sickness [5], radiation-induced tissue injury [6], ischaemic wounds [7], thermal burns [8] and refractory osteomyelitis [9]. HBO appears to work by increasing the partial pressure of oxygen to a level that promotes the formation of collagen matrix and angiogenesis, thus promoting healing in wounds and necrotic tissue [10]. HBO is also known to increase the generation of oxygen free radicals and thus there is a potential that it may increase oxidative stress, even during administration for these therapeutic indications [11].

This study aimed to measure plasma IsoFs and F_2-IsoPs in patients receiving hyperbaric therapy to treat osteonecrosis secondary to radiation therapy. Our hypothesis was that IsoFs would continue to rise with increasing oxygen pressures in contrast to F_2-IsoPs whose synthesis would be reduced. The novelty of this study lies in the measurement of whole body oxidative stress using a reliable biomarker of lipid oxidation sensitive to changes in oxygen concentration, in a setting where patients are being exposed to extremely high concentrations of oxygen such as occurs during HBO therapy.

2. Methods

Patients were recruited from the Hyperbaric Unit at Fremantle Hospital after being identified by hyperbaric physicians and anaesthetists as being suitable for the study. Institutional ethics was obtained from the South Metropolitan Area Health Service and all participants gave signed informed consent. Patients were 18 years or older and were booked to receive hyperbaric therapy to treat osteonecrosis secondary to radiation therapy. They were excluded from the study if they had active systemic infection/inflammatory disease or any risk factors for barotrauma. The trial was prospectively registered with the Australia and New Zealand Clinical Trials Registry (Registration Number -ACTRN12612000336886)

2.1. Study design

On the day of HBO therapy an intravenous cannula was inserted into the antecubital vein to obtain blood samples for the measurement of plasma F_2-IsoPs, IsoFs, blood gases and haemoglobin. Seven blood (2 ml) samples were collected over the treatment period of 119 min (Fig. 1). Blood was collected at the following times: (T1) prior to HBO therapy at normal air pressure; (T2) after 15 min and (T3) after 55 min at 2.4 atm and 100% O_2; (T4) after 60 min HBO treatment with a 5 mins air break; (T5) after 100 mins HBO treatment, and at the end of HBO treatment after 119 min (T6). A further blood sample (T7) was collected at 149 min corresponding with 30 min after cessation of HBO therapy.

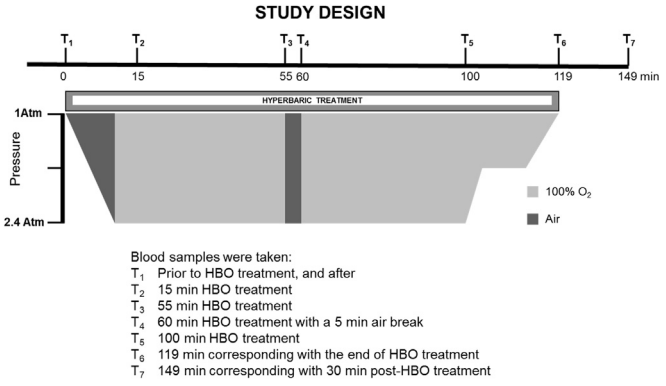

Fig. 1. The study design showing the timing of blood samples during hyperbaric oxygen treatment.

Blood samples for measurement of IsoFs and F_2-IsoPs were collected into cold tubes containing EDTA, reduced glutathione and butylated hydroxytoluene, centrifuged at 1500*g* and the plasma stored at −80 °C [12].

Venous blood gas samples were drawn at each time point into 80 IU of electrolyte-balanced heparin solution (PICO 50®, Radiometer Medical ApS, Copenhagen) and immediately underwent blood gas, electrolyte, haemoglobin and lactate analysis on a Radiometer ABL 800FLEX automated point-of-care device (Radiometer Medical ApS, Copenhagen). Venous blood oxygen content was calculated from the equation CvO2 (ml/L) = (SvO2 x Hb × 1.34) + 0.0031(PvO2), where SvO2 represents venous blood oxygen saturation (%), Hb is the haemoglobin concentration (g/L) and PvO2 is the venous oxygen partial pressure (mmHg).

2.2. Measurement of plasma F_2-IsoPs and IsoFs

F_2-IsoPs and IsoFs were measured by gas chromatography-mass spectrometry (GC-MS) using a modification of our previously reported method [13]. Briefly, internal standard (5 ng, 15-F_{2t}-IsoP-d_4) was added to plasma (200 µl) and samples were hydrolysed with KOH in methanol, acidified and applied to pre-washed Certify II cartridges (Varian). The cartridges were washed with methanol/water (1:1) and then hexane/ethyl acetate (75:25). The F_2-IsoPs and IsoFs were eluted with ethyl acetate/methanol (90:10), dried and derivatised and quantitated by GC-MS monitoring ions at *m/z* 569, 573 and 585, for F_2-IsoPs, 15-F_{2t}-IsoP-d_4 and IsoFs, respectively. The intra-assay and inter-assay coefficients of variation were 8% and 5.6% for plasma F_2-IsoPs, and 9%, and 10% for IsoFs.

2.3. Statistics

The effects of hyperbaric treatment on plasma F_2-IsoPs and IsoFs were examined over time after log transformation using longitudinal linear mixed effects regression in STATA®. Our experience of longitudinal analysis in a surgical setting has shown that over the period between pre-anaesthesia and post-anaesthesia it is possible that we find non-linear longitudinal changes which are typically U-shaped or N-shaped (see an example in Fig. 2(a)). Such a pattern can produce incorrect results in a linear model. To detect this effect each of the models was combined with a test of linearity which is based upon the pattern of residuals [14]. A P value < 0.05 was taken to indicate statistical significance.

3. Results

Twelve patients (9 men and 3 women) completed the study. They had a median age of 63yrs (range 32–82 yrs). The reasons for HBO therapy included radiation cystitis (n = 6), dental extraction or predental clearance (n = 4) and osteoradionecrosis prophylaxis. (n = 2). The pre-existing conditions were prostate cancer (n = 5), craniopharyngioma (n = 1), non-Hodgkins lymphoma and brain tumor (n = 1), oral carcinoma (n = 1), dermatofibrosarcoma (n = 1), pleomorphic adenoma (n = 1), parotid B-cell lymphoma (n = 1), squamous cell carcinoma of the tongue (n = 1). One patient was not taking any medication but the other patients were taking between 1 and 8 medications. Medications included NSAIDS (n = 6), corticosteroids (n = 2), statins (n = 5), proton pump inhibitors (n = 2), angiotensin converting enzyme inhibitors or angiotensin 2 blockers (n = 3), anticonvulsants (n = 1), bromocriptine (n = 1), serotonin re-uptake inhibitors (n = 1), thyroxin (n = 1), testosterone (n = 1), and fish oil (n = 1).

During hyperbaric treatment venous oxygen saturation (SvO2) increased from 56% at baseline to above 85% saturation after 100 min of therapy ($P < 0.0001$) (Fig. 2a). PvO_2 increased during the period of