HIF-1 Sensor in Detecting Hypoxia Tolerance at High Altitude

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INTRODUCTION: An episode of prolonged exposure to high altitude can cause hypoxia and have significant health consequences. In people with a high-altitude disorder, the body reacts by producing a protein called hypoxia-inducible factor (HIF), which triggers a series of physiological changes and serves a central role in the hypoxia response. Its activity is regulated by the oxygen-dependent degradation of the HIF-1α protein (HIF-1A gene). Therefore, the effects of low oxygen tension in high altitude were explored using fluorescent sensors of hypoxia.

METHODS: The development of the sensor provided more sensitivity for detecting hypoxia by generating a calibration of optimized parameters such as reagent concentrations, reagent volumes, and device dimensions.

RESULTS: There is a high sensitivity and specificity in detecting the changes of HIF-1α protein hypoxia using the feasibility hypoxia test. This would enable point-of-care (POC) testing and individual self-administration, resulting in faster and more accurate results that can be used for a robust diagnostic approach and enhanced health surveillance, particularly in high-altitude exposure.

KEYWORDS: HIF-1a (hypoxia-inducible factor 1alpha), hypoxia, high altitude, HIF-1 sensor.

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Aircrews exposed to high altitude with longer durations are at a higher potential risk for detrimental health consequences. The altitude and other environmental features are of concern for aircraft safety.[10] For safety reasons, proper acclimatization is important for those traveling to high altitudes. While the effect is most dramatic at altitudes greater than 8000 ft (2438 m) above sea level, it becomes noticeable even at 5000 ft (1524 m) above sea level.[5,6] Among other important changes (e.g., decreases in temperature and ambient humidity), the defining environmental feature at high altitude is a drop in barometric pressure, which causes a decrease in the partial pressure of oxygen at every point along the oxygen transport cascade from ambient air to cellular mitochondria.[1,11] Subsequently, there is also a decrease in the Po_2 at every point along the oxygen transport cascade from inspired air to the alveolar space, arterial blood, tissues, and venous blood. The higher the elevation attained and the longer the duration of spaceflight, the greater the drop in Po_2 in the human body. These declines in oxygen tensions trigger a variety of physiological responses in the cardiovascular system over a period of minutes to weeks after the initial altitude hypoxia exposure, all of which enable the individual to adapt to the hypoxic environment. Indeed, short-term altitude exposure can directly or indirectly affect the vascular tone of systemic resistance vessels and enhances ventilation and sympathetic activity through the activation of peripheral chemoreceptors.[12]

Ascent to high altitude is associated with physiological responses that counter the stress of hypobaric hypoxia by increasing oxygen delivery and altering tissue oxygen utilization via metabolic modulation.[3] At the cellular level, the transcriptional response to hypoxia is mediated by the

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hypoxia-inducible factor (HIF) pathway and results in promotion of glycolytic capacity and suppression of oxidative metabolism.[2] Hypoxia inducible factor-1 (HIF-1) plays a key role in oxygen homeostasis by facilitating oxygen supply to the tissues under hypoxic conditions, as during acclimatization to hypobaric hypoxia or in the hypoxemia or inflammation molecular response. HIF-1 is found in almost all body tissues. Under normoxic conditions, it is degraded through hydroxylation, but it does not undergo degradation in the presence of hypoxia.

Retrospective studies conducted after the Second World War give an account of a significant number of unexplained military aircraft accidents that were suspected to be due to hypoxia.[4,6,7] Detection of possible hypoxia exposures during postmortem investigation of aircraft accidents has implications for determining flight safety. A study conducted by Tripathi et al. from 1986 to 1995 in Army Aviation helicopters flying high-altitude sorties revealed 29 accidents, and hypoxia was a contributing factor in 24% of those cases.[9] Pilot incapacitation attributable to hypoxia has been confirmed as the cause of the crash of IAF MiG 29 at Sirsi, Karnataka, on April 11, 2002.[8]

In this study, we decided to explore the effectiveness of the hypoxia sensor, which could potentially be used as a diagnostic tool for detecting physiological changes due to hypoxia at high altitude during a long spaceflight.

METHODS

Lateral flow immunoassay (LFIA) is a qualitative chromatography that provides very simple, rapid, on-site detection of a target and serves as a portable analytical platform that specifically detects antigens or antibodies. LFIAs are typically composed of a sample pad, a conjugated pad, a nitrocellulose membrane, and an absorbent pad, as shown in **Fig. 1**.

Since the hypoxia marker has been identified as HIF-1a, the protein was examined using the "Dot Blot" approach known as Dot Blot analysis. This is a technique for detecting, analyzing, and identifying proteins; it is similar to the western blot technique except that protein samples are not separated electrophoretically—instead, they are spotted directly onto the membrane or paper substrate through circular templates. The concentration of HIF-1a protein was purchased and detected using monoclonal antibodies against HIF-1a.

RESULTS

The feasibility of HIF-1a is shown expressed in athletes after moderate exercise intensity. This research demonstrated that an effective hypoxia tolerance device is achieved by using the sensors. By accepting HIF-1a as a protein marker, the assay proved the presence of hypoxia signals in human subjects

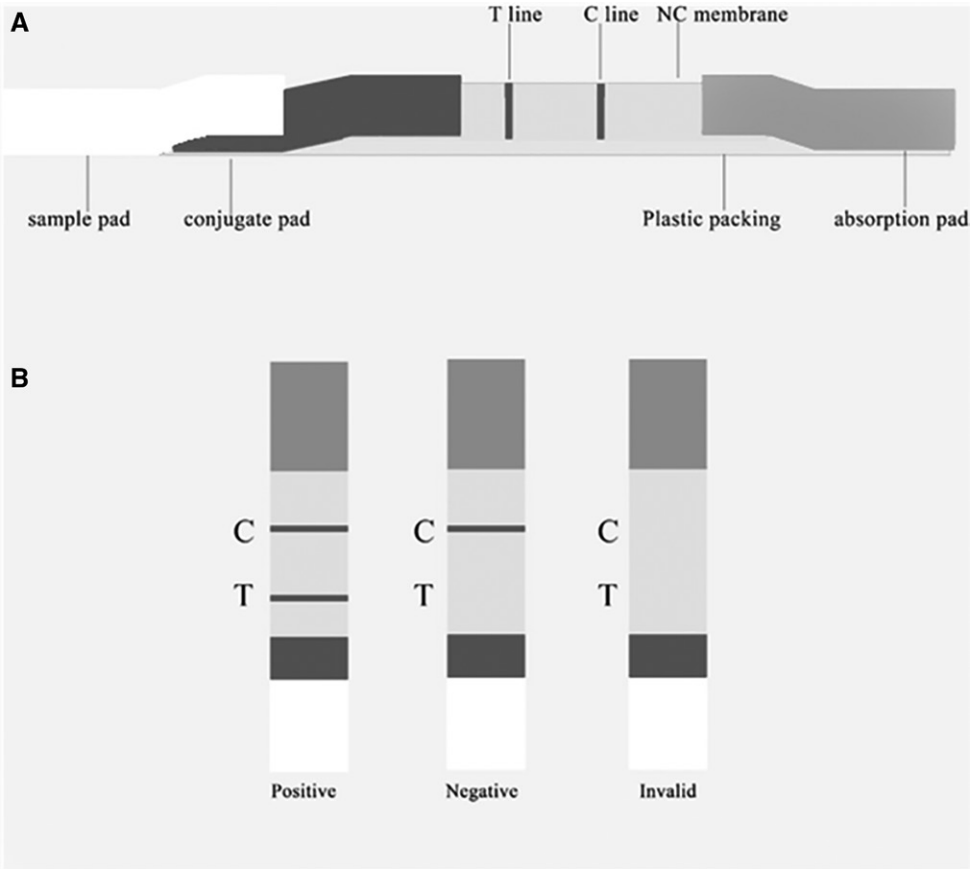

Fig. 1. Images and design of the lateral flow strip.

after moderate exercise intensity. Based on the compression feature, a sensor for the hypoxia tolerance device was produced. A desirable sensor should not only be highly sensitive and selective, but also capable of directly viewing results on the device, making it portable. Such devices could be utilized in settings with limited resources, close to the patient, and outside of a laboratory.

DISCUSSION

Monitoring HIF levels within cells and tissues provides a measure of the extent of hypoxia and hypoxic gradients. Hence, detection of hypoxia often relies on strategies for the detection of HIF protein expression. There are several techniques to detect hypoxia by targeting HIF protein, including western blot, immunoassay (e.g., ELISA, Luminex), immunohistochemistry, and flow cytometry. These techniques require specialized equipment and complex data analysis. It also takes time to get the results. Due to the importance of early detection of hypoxia to avoid acute symptoms and the limitation of detection by using the current techniques, we developed lateral-flow assays to detect hypoxia early via targeting the HIF protein.

In conclusion, this study exploited new technology to develop a hypoxia sensor to detect the risk of developing hypoxia, which has been recognized as one of the foremost physiological threats at high altitude. The development of strip-based detection enables the use of an enzyme-linked assay in a lateral-flow device to provide a more sensitive, precautionary screening tool for detecting the risk of and preventing hypoxia for aircrews.

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