Biomedicine & Pharmacotherapy 86 (2017) 502–513

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Ascorbic acid, but not dehydroascorbic acid increases intracellular vitamin C content to decrease Hypoxia Inducible Factor -1 alpha activity and reduce malignant potential in human melanoma

Adam P. Fischer, Sarah L. Miles*

Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, One John Marshall Drive, Huntington, WV 25755, United States

ARTICLE INFO

Article history:
Received 19 August 2016
Received in revised form 9 December 2016
Accepted 14 December 2016

Keywords:
Melanoma
Hypoxia inducible factor-1 alpha
Ascorbic acid
Dehydroascorbic acid
SVCT2

ABSTRACT

Introduction: Accumulation of hypoxia inducible factor-1 alpha (HIF-1α) in malignant tissue is known to contribute to oncogenic progression and is inversely associated with patient survival. Ascorbic acid (AA) depletion in malignant tissue may contribute to aberrant normoxic activity of HIF-1α. While AA supplementation has been shown to attenuate HIF-1α function in malignant melanoma, the use of dehydroascorbic acid (DHA) as a therapeutic means to increase intracellular AA and modulate HIF-1α function is yet to be evaluated. Here we compared the ability of AA and DHA to increase intracellular vitamin C content and decrease the malignant potential of human melanoma by reducing the activity of HIF-1α.

Methods: HIF-1α protein accumulation was evaluated by western blot and transcriptional activity was evaluated by reporter gene assay using a HIF-1 HRE-luciferase plasmid. Protein expressions and subcellular localizations of vitamin C transporters were evaluated by western blot and confocal imaging. Intracellular vitamin C content following AA, ascorbate 2-phosphate (A2P), or DHA supplementation was determined using a vitamin C assay. Malignant potential was accessed using a 3D spheroid Matrigel invasion assay. Data was analyzed by One or Two-way ANOVA with Tukey's multiple comparisons test as appropriate with $p < 0.05$ considered significant.

Results: Melanoma cells expressed both sodium dependent vitamin C (SVCT) and glucose (GLUT) transporters for AA and DHA transport respectively, however advanced melanomas responded favorably to AA, but not DHA. Physiological glucose conditions significantly impaired intracellular vitamin C accumulation following DHA treatment. Consequently, A2P and AA, but not DHA treated cells demonstrated lower HIF-1α protein expression and activity, and reduced malignant potential. The ability of AA to regulate HIF-1α was dependent on SVCT2 function and SVCT2 was not significantly inhibited at pH representative of the tumor microenvironment.

Conclusions: The use of ascorbic acid as an adjuvant cancer therapy remains under investigated. While AA and A2P were capable of modulating HIF-1α protein accumulation/activity, DHA supplementation resulted in minimal intracellular vitamin C activity with decreased ability to inhibit HIF-1α activity and malignant potential in advanced melanoma. Restoring AA dependent regulation of HIF-1α in malignant cells may prove beneficial in reducing chemotherapy resistance and improving treatment outcomes.

© 2016 Elsevier Masson SAS. All rights reserved.

1. Introduction

The incidence of melanoma, a malignancy derived from pigment producing melanocytes, has continually risen over the past 30 years and accounts for 75% of skin cancer deaths. In 2016 an estimated 76,000 new cases of skin melanoma will be diagnosed in the United States, contributing to over 10,000 fatalities by year's end [1]. Most cutaneous melanomas can be readily cured by

Abbreviations: BRAF, Murine sarcoma viral (v-raf) oncogene homolog B1; HIF-1α, Hypoxia inducible factor-1 alpha; PHD, prolyl hydroxylase; FIH, Factor Inhibiting HIF; AA, Ascorbic acid; A2P, Ascorbate 2-phosphate; DHA, Dehydroascorbic acid; EDHB, Ethyl 3, 4-dihydroxbenozoate; SVCT, Sodium dependent vitamin C transporter; GLUT, Glucose transporter; NAC, N-acetyl cysteine; ChCl, Choline chloride.

* Corresponding author.
E-mail addresses: fischer35@marshall.edu (A.P. Fischer), kittlaus1@marshall.edu (S.L. Miles).

http://dx.doi.org/10.1016/j.biopha.2016.12.056
0753-3322/© 2016 Elsevier Masson SAS. All rights reserved.

surgical excision [2,3], however once disseminated, metastatic melanoma is highly aggressive and difficult to treat. Five year survival of melanoma patients declines from 98 to 17% following metastasis to a distant site [1]. Even though clinical responses have seen some improvement through the use of immunotherapy, poor patient prognosis, attributed to chemotherapy resistance, highlights the need for alternative or adjuvant treatment options to improve survival in melanoma patients.

Melanoma oncogenesis is predominantly driven by the acquisition of BRAF mutations; 50% of all melanomas contain either a V600E or V600D BRAF mutant [4]. This mutation results in the constitutive activation of signaling pathways leading to unchecked cell proliferation, invasion, and metastasis. Constitutive BRAF activation is also known to contribute to elevated gene expression of hypoxia inducible factor-1 alpha (HIF-1α) [5], the oxygen responsive subunit of the HIF-1 transcription factor. HIF-1α activity in malignant tissue contributes to increased expression of proteins that drive melanoma cell motility and invasion [6]. Elevated expression of HIF-1α protein is widespread in malignant tissue, including melanoma [7], and has been linked to poor patient outcomes in a variety of cancers (reviewed in [8]). Ascorbic acid (AA; reduced vitamin C) is an essential cofactor for Fe II/2-oxoglutarate dioxygenase enzymes including the prolyl hydroxylase (PHD1-3) and factor-inhibiting HIF (FIH) hydroxylase enzymes that regulate HIF-1α protein stability and transcriptional activity respectively [9,10]. Plasma AA concentration in healthy individuals is typically between 40 and 80 μM [11]. Interestingly, cancer patients, including those with melanoma [12], have been observed to have below normal levels of plasma AA [13–16]. Likewise, tumor tissue has been found to contain decreased intracellular AA compared to paired non-transformed tissue from the same patient [17]. The degree of AA deficiency in malignant tissue also correlates with increased tissue accumulation of HIF-1α protein and with tumor stage [17,18], suggesting that inadequate intracellular AA may contribute to the development or progression of a malignant phenotype. The majority of current studies investigating AA as an anti-cancer therapy utilize I.V. administered mega or high dose (>1 mM) AA supplementation to induce cytotoxic cell death, however the potential benefit and use of AA supplementation at physiological concentrations to restore or support its cofactor functions in malignant cells has remained largely unexplored and uncharacterized. Recently, we reported that supplementation of human metastatic melanoma cells with physiological concentrations (10–100 μM) of ascorbic acid inhibits both normoxic and hypoxia-mimetic induced protein accumulation and transcriptional activity of HIF-1α, and reduces the malignant potential of these cells [19,20], emphasizing the functional importance of physiological levels of AA in malignant cells. I*n vivo* studies using Gulo −/− mice (a model of human AA dependency) inoculated with murine melanoma cells demonstrate that AA supplementation can decrease tumor accumulation of HIF-1α [21] and decrease tumor volume [21–23]. Furthermore, tumor ascorbate levels inversely correlated to the expression of HIF-1 target genes [21], providing further support for initiating the use of AA as an adjuvant cancer therapy.

Dietary vitamin C[1] is comprised of both reduced AA and the fully oxidized form, dehydroascorbic acid (DHA). The ability of AA and DHA to provide equivalent intracellular vitamin C activity has been controversial for decades with conflicting reports on the ability of DHA intervention to prevent or treat scurvy in animal and human subjects [24–27]. Recently, McCarty [28] made the speculation that DHA would be a more effective cancer therapy than AA. The rationale for this idea was that advanced malignancies, particularly those with elevated HIF activity, often overexpress glucose transporters (GLUTs), a common observation in Warburg metabolism [29]. Interestingly, DHA uptake is facilitated by GLUTs, therefore elevated GLUT1 expression, which is a known HIF-1 target gene, would support increased DHA entry into malignant cells [28]. DHA itself does not have any biological activity or act as an enzyme cofactor, however, once transported into the cell it is reduced to AA in the cytosol, providing the functional form of vitamin C (Fig. 1) [11]. To our knowledge, the capacity of using DHA rather than AA, as an effective clinical source for increasing intracellular AA levels has not been evaluated in malignant cells.

There are several physiological factors that may impair the delivery of adequate vitamin C to melanoma cells via AA or DHA supplementation. Some of these include the expression and subcellular localization of sodium dependent vitamin C transporters (SVCT1 & 2) and glucose transporters (GLUTs), which transport either AA or DHA respectively. DHA also competes with glucose for entry into the cell through the GLUT transporters and is known to be unstable at physiological pH [30]. Therefore, the effect of glucose competition on DHA uptake, poses a relevant concern for the use of DHA in the clinical setting as a means to promote intracellular vitamin C accumulation and warrants evaluation. The objective of this study was to compare the suitability of AA vs. DHA as a potential adjuvant cancer therapy to reduce the malignant potential of melanoma cells by increasing intracellular vitamin C content and subsequently restoring regulation of aberrant normoxic HIF-1α protein accumulation and activity in human melanoma cells.

2. Methods

2.1. Cell culture and reagents

WM3211, SbCl2, WM3248, WM1366, WM239A, and WM9 melanoma cell lines were a generous gift from Meenhard Herlyn's lab at the Wistar Institute (University of Pennsylvania). Human Epidermal Melanocytes, neonatal, lightly pigmented (HEMnLP) were purchased from Life Technologies. All cells were cultured in a humidified incubator with 5% CO_2/95% air at 37 °C. SbCl2 cells were cultured in MCDB 153 media (Sigma) supplemented with 2% fetal bovine serum (FBS), 5 μg/mL insulin (Sigma), 1.68 mM $CaCl_2$, and 1% penicillin/streptomycin. WM3211 cells were cultured similarly expect for 5% FBS without $CaCl_2$. WM3248, WM1366, WM239A, and WM9 cells were cultured in standard RPMI 1640 media (Gibco; 1 g/L glucose as indicated in text) supplemented with 10% FBS and 1% penicillin/streptomycin. HEMnLP cells were cultured in Medium 254 (ThermoFisher) supplemented with Human Melanocyte Growth Supplement (HMGS; ThermoFisher) and 1% gentamicin/amphotericin B. L-Ascorbic Acid (AA), Dehydroascorbic acid (DHA), L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate (A2P), Ethyl 3, 4-dihydroxbenozoate (EDHB), and Cobalt Chloride ($CoCl_2$) were purchased from Sigma. Glucose Transporter Inhibitor III; STF-31 was purchased from EMD Millipore.

[1] Typically, it is convention to use the terms vitamin C and ascorbic acid (AA) interchangeably when discussing human physiology. However, in this manuscript we are evaluating two distinct forms of vitamin C, reduced vitamin C (i.e. AA) and oxidized vitamin C, dehydroascorbic acid (DHA). In the present study the term vitamin C is used to describe both forms of ascorbate (AA or DHA), total ascorbate (the summation of AA and DHA), or when the form of vitamin C cannot be distinguished because of assay limitations, thus the terms vitamin C and AA are not necessarily interchangeable.

Download English Version:

https://daneshyari.com/en/article/5553566

Download Persian Version:

https://daneshyari.com/article/5553566

Daneshyari.com