Commentary

Antitumoral effects of pharmacological ascorbate on gastric cancer cells: GLUT1 expression may not tell the whole story

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Abstract

The recently reported results of Lu *et al.* (Theranostics. 2018; 8: 1312-26. doi:10.7150/thno.21745) – highlighting GLUT1 expression as a marker for sensitivity of gastric cancer cells to therapeutic doses of ascorbate – are discussed in the light of additional factors potentially affecting the underlying processes, such as the concomitant expression of membrane gamma-glutamyltransferase activity, the resistance of cancer cells to oxidative injury and other known biomarkers.

Key words: Ascorbic acid; Gastric cancer; GLUT1; gamma-glutamyltransferase.

Introduction

Ascorbic acid (AA) has been proposed as an anticancer treatment during several decades, since the seminal observation by Cameron & Pauling that supplementation of the vitamin would often allow a remarkable prolongation of survival of terminal cancer patients [1]. Numerous studies have been published to date largely confirming the beneficial action of high dose ascorbate, alone or in association with other micronutrients, or as a means to sensitize cancer cells to the effects of other recognized genotoxic chemotherapeutics.

It was originally thought that AA might be involved in host resistance to expansion of the disease as it would support collagen biosynthesis, thus helping maintain the resistance of the ground substance to malignant invasive growth [2]. On the other hand, with the exploration of biochemical mechanisms underlying the phenomenon it has become increasingly clear that cytostatic/cytotoxic effects of ascorbate are prevailing, and are correlated with prooxidant reactions. The prooxidant activity of AA is well known, and in selected conditions can predominate over its antioxidant properties [3, 4]. In particular, it has been documented that AA can give rise to increased steady-state levels of reactive oxygen species (ROS), including superoxide and hydrogen peroxide, which can produce an increase in the redox-active labile iron pool of cancer cells. Iron metabolism is in fact often altered in cancer cells, with upregulation of iron uptake and downregulation of export and storage pathways. The cytotoxic effect of AA can be thus explained as the result of iron-dependent prooxidant damage involving cell membranes, mitochondria and DNA [5, 6].

GLUT1 expression as a marker of sensitivity to AA

A recent article by Lu et al. in this Journal confirmed the therapeutic potential of high-dose ascorbate, and added further intriguing insights [7]. The authors demonstrated that gastric cancer cells with high GLUT1 expression were more sensitive to ascorbate treatment than cells with low GLUT1 expression. AA was shown to deplete intracellular

glutathione, generate oxidative stress and induce DNA damage. The combination of pharmacological AA with genotoxic agents (oxaliplatin, irinotecan) was also investigated, showing a synergistical inhibition of tumor growth *in vivo.* GLUT1 expression may serve thus as a marker for sensitivity of gastric cancer cells to AA treatments.

Extracellular AA oxidation: the role of gamma-glutamyltransferase

One critical aspect of the matter however was not discussed, *i.e.*, the fact that facilitation of ascorbate uptake by cells through the glucose transporter protein, GLUT1 (as well as GLUT3) can only occur after extracellular AA is oxidized to dehydroascorbic acid (DHA: a molecule structurally similar to glucose). Once within cells, DHA is then immediately reduced to AA by both chemical and protein mediated processes [8]. Indeed, it was shown that tumor cells can spontaneousy obtain vitamin C from the medium by inducing the oxidation of AA to DHA, and such oxidation was ascribed to NADPH oxidase activities present in non-malignant stromal cells (*e.g.* phagocytes, fibroblasts, endothelial cells) [9]. An endogenous production of ROS has been observed in several cancer cell types including melanoma, where it likely ensues from prooxidant reactions taking place during melanogenesis [10]. On the other hand, studies performed in our own laboratories allowed to describe another, potentially crucial source of prooxidants capable of oxidizing AA to DHA at the surface of cancer cells, *i.e.* membrane gamma-glutamyltransferase (GGT) activity. We have documented that membrane GGT activity is an autocrine source of ROS and other free radicals, capable of promoting prooxidant reactions at the cellular surface and in the microenvironment [11]. High levels of GGT activity are often expressed in many human neoplasms, both primitive and metastatic (carcinomas of ovary, colon, liver; sarcoma; leukemias; melanoma; reviewed in [12]). We showed that GGT-expressing cancer cells can exploit this activity in order to oxidize extracellular AA and subsequently uptake the resulting DHA [13], similarly to what was described for activated neutrophils following the onset of respiratory burst [14].

Other factors involved in AA sensitivity

A recent study has highlighted the expression of GGT in gastric cancer as well, and proposed tumor GGT levels as a poor prognostic factor as it was associated with lymph node metastasis and progression through EMT, KRAS, SRC and PKCA pathways [15]. Interestingly, others demonstrated the involvement of Ras pathway in the oxidative

stress-induced activation of GGT in colon carcinoma cells [16]. Also, a recent study showed that KRAS and BRAF mutants presented with an increased expression of GLUT1. These cells were more sensitive to ascorbate cytotoxicity, mediated through DHA-induced inhibition of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and NAD^+ depletion, resulting in a decreased glycolytic flux [17]. Thus, a combination of GLUT1 and GGT expression, as well as KRAS mutation, could improve the identification of cancer patients with enhanced sensitivity to AA. As recently rewieved [18] and mentioned above, AA toxicity was also related to the intracellular levels of iron (the so-called labile iron pool, LIP), at least in glioblastoma and NSCLC cell lines [6]. Others showed that Ras (and c-Myc)-dependent pathways were also involved in the increase of LIP [19, 20].

Actually, several factors may concur to modulate ascorbate citotoxicity, and the efficacy of potential biomarkers in predicting AA sensitivity likely depends on the individual cancer cell types considered. In melanoma cells, we found that increased GGT levels can induce a higher resistance against oxidative stress due to an increased activity of catalase. This phenomenon could in principle protect cancer cells from ascorbate-dependent (prooxidant) cytotoxic effects [21]. Indeed, it was reported that tumor levels of catalase activity could predict which cancers would respond to pharmacological AA [22]. In our melanoma model, increased catalase stability and activity – associated with increased p38 phosphorylation – was interpreted as the result of a persistent, low-level oxidative stress induced by GGT expression [23]. Anyway, although GGT-overexpressing cells were resistant to oxidative stress, in our hands the prooxidant action of ascorbate might still be exploited in order to enhance the cytotoxicity of another prooxidant agent, arsenic trioxide [23]. Several recent studies focused on combination therapies aiming at overcoming the antioxidant resistance of tumors expressing high catalase activities, e.g. [18, 22, 24]. In this perspective, the inhibition/modulation of GGT-dependent pathway(s) involved in the observed increase in catalase activity could be proposed as a further means for enhancing the therapeutic potential of ascorbate.

Conclusions

In the light of the data discussed above, it can be speculated that expression levels of GLUT1 *and* GGT – together with Ras mutation – could be *jointly* investigated, as the combination of high levels of these biomarkers might identify neoplasms with even higher sensitivity to treatments with pharmacological

ascorbate. In particular, GGT expression appears to be associated with more aggressive forms, for which the identification of effective treatments would be of even higher value.

Abbreviations

AA: ascorbic acid; DHA: dehydroascorbic acid; ROS: reactive oxygen species; GGT: gamma-glutamyltransferase.

Competing Interests

The authors have declared that no competing interest exists.

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