SIGNIFICANCE OF ASCORBATE IN TRANS-PLASMA MEMBRANE ELECTRON TRANSPORT IN HEALTH AND DISEASE OF HUMANS: REVIEW

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INTRODUCTION

Transplasma membrane electron transport (t-PMET) has been established in the year 1960s. This system (t-PMET system) transfer electron across the plasma membrane, which results in the net reduction of extracellular oxidants (e.g., ferricyanide) at the cost of intracellular reductants such as NADH and ascorbate. Ascorbate (vitamin C), helps in the protection of organism against a variety of oxidative agents. Oxidation of ascorbate takes place in two one-electron steps, the first step results in the Ascorbate Free Radical (AFR) formation. AFR can be oxidized further to produce dehydroascorbic acid (DHA) and also two molecules of AFR disproportionate to form one DHA and one ascorbet molecule. In humans, NAD(P)H- and NADH-dependent system have been distinguished. Recent finding suggest that transplasma membrane ascorbate/dehydroascorbate cycling enhance NTBI reduction and uptake by human erythroleukemia (K562) cells. By this phenomenon cell can respond to change in the redox microenvironment which is responsible for regulating several biological functions such as cell metabolism, proton pumping, and activity of ion channels, growth and death. This review will give an update on functional significance of ascorbate in t-PMET and emphasis on its correlation to some harmful diseases, such as cancer, abnormal cell death, cardiovascular diseases, aging, obesity, metabolic syndrome etc. and genetically linked pathologies.

Keywords: Dehydroascorbate, ascorbate free radical, K562 cells, vitamin.

ABSTRACT

Trans-Plasma membrane electron transport (t-PMET) has been established in the year 1960s. This system (t-PMET system) transfer electron across the plasma membrane, which results in the net reduction of extracellular oxidants (e.g., ferricyanide) at the cost of intracellular reductants such as NADH and ascorbate. Ascorbate (vitamin C), helps in the protection of organism against a variety of oxidative agents. Oxidation of ascorbate takes place in two one-electron steps, the first step results in the Ascorbate Free Radical (AFR) formation. AFR can be oxidized further to produce dehydroascorbic acid (DHA) and also two molecules of AFR disproportionate to form one DHA and one ascorbet molecule. In humans, NAD(P)H- and NADH-dependent system have been distinguished. Recent finding suggest that transplasma membrane ascorbate/dehydroascorbate cycling enhance NTBI reduction and uptake by human erythroleukemia (K562) cells. By this phenomenon cell can respond to change in the redox microenvironment which is responsible for regulating several biological functions such as cell metabolism, proton pumping, and activity of ion channels, growth and death. This review will give an update on functional significance of ascorbate in t-PMET and emphasis on its correlation to some harmful diseases, such as cancer, abnormal cell death, cardiovascular diseases, aging, obesity, metabolic syndrome etc. and genetically linked pathologies.

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BIOCHEMISTRY OF ASCORBATE

L-ascorbic acid (C_6H_8O_6) is the trivial/common name of Vitamin C. The chemical name of ascorbate is 2-oxo-L-threo hexono-1,4-lactone-2,3-enediol. L-ascorbic and dehydroascorbic acid are the major dietary forms of vitamin C. The monovalent ascorbate anion undergoes sequential one-electron oxidations under physical condition of pH, temperature and oxygen tension [5]. The first oxidation product is relatively long-lived and electrochemically stable ascorbet free radical (AFR; also known as semi- or mono-dehydroascorbate; \( E'_0 = +330 \text{mV} \)). The first oxidation step of ascorbate requires relatively low levels of circulating redox-active transition metals, such as iron and copper [2]. AFR is unreactive with dioxygen dissimilar to other free radicals and it tends to decay mainly by disproportionation, the formation of AFR takes place by reaction of ascorbate with reactive radical species which tends to inhibit free radical-induced oxidative chain reactions and it is irreversible reaction; and rapidly AFR reduced back to ascorbate. Further monoelectronic oxidation of AFR produced DHA (\( E'_0 = +210 \text{mV} \)) in the presence of mild oxidant such as ferricyanide and /or NTBI species [4]. In the absence of oxidants, two AFR molecules rapidly oxidized to form one ascorbate and one DHA molecule (fig.1). Though oxidation (or disproportionation) of AFR to DHA requires two-electron reducing capacity of ascorbate, DHA is a structurally labile species which rapidly undergoes an irreversible hydrolytic reaction also known as ring-opening reaction to form 2,3-diketogulonic acid in plasma with a half-life of several minutes [4].

These reactions mainly require enzymes, e.g. glutaredoxin, thioredoxin reductase, or AFR reductases, also chemical reduction by glutathione alone has been described. When extracellular oxidation occurs, then DHA reduction takes place into the cell. In case of erythrocytes, AFR and DHA can be reduced extracellularly by redox enzymes present in the plasma membrane, which require intracellular NADH as a source of reducing equivalents [6]. These are evidence of alternative pathway for the reduction of extracellular ascorbate free radicals in the erythrocyte (fig.2). The intracellular ascorbate provides the reducing equivalents for the reaction but not NADH, which utilized a transmembrane redox enzyme. This reaction is similar to the redox process present in the adrenal chromaffine granules. There is possibility of another, similar, erythrocyte redox system responsible for the reduction of AFR. On the other hand, it is suggested that electrons can be transported to the membrane by small lipid...
soluble molecules like á-tocopherol and coenzyme Q\(^7\). DHA degradation results in a complete loss of the vitamin from human systems—it is a point which is particularly pertinent in the case of species which lacks gulono-á-lactone oxidase activity. In order to overcome the loss of ascorbate, the vitamin must be retained predominantly in the two-electron reduced form (i.e. ascorbate) in both intracellular and extracellular biological fluids. This observation implies that, human cells possess several conservative reduction mechanisms for maintaining both intra- and extracellular ascorbate. Even cultured cells, which are supplemented with artificial standard culture conditions, maintain an extraordinary ability for ascorbate regeneration\(^6\).

**ASCORBATE-STIMULATED PLASMA MEMBRANE FERRICYANIDE REDUCTASE**

tPMET activity is present in human erythrocytes that utilizes intracellular ascorbate which acts as major electron donor for reduction of extracellular ferricyanide. After increasing intracellular ascorbate by dehydroascorbate, stimulation of the plasma membrane ferricyanide reductase activity takes place\(^8\). By the addition of ascorbate oxidation the stimulation of ferricyanide reductase activity is not affected (all extracellular ascorbate is oxidized to form DHA and inhibits direct reduction of ferricyanide by ascorbate), therefore intracellular ascorbate acts as an electron donor for reduction of extracellular ferricyanide (Lane et al., data not shown). Direct addition of ascorbate could not reproduce the stimulation of reductase activity; hence these cells do not express significant levels of sodium-ascorbate co-transporters (SVCTs)\(^9\).

**CELLULAR DHA UPTAKE**

Maximum human cells are able to maintain intracellular ascorbate concentration that is remarkably higher e.g. up to 30-fold in some cases in comparison to the extracellular fluid or plasma. Although most of the cells maintain outward-facing concentration gradient by SVCT-mediated ascorbate import\(^4\), low-affinity, high-capacity GLUTs is also a significant contributor to facilitate the diffusion of DHA. An inward-facing DHA gradient is maintained with respect to DHA through rapid reduction of imported DHA back to ascorbate by the cells; ascorbate is poor substrate for GLUT-mediated transport\(^5\). Elevated level of intracellular ascorbate is seen after loading the K562 cells with DHA. This is inhibited by cytochalasin B. GLUTs is responsible for DHA uptake by K562 cells which is evidenced by two pharmacological observations, the accumulation of intracellular ascorbate is response to extracellular DHA inhibited by: i) low micro molar concentration of cytochalasin B, but not the structural analog dihydrochalasin B, the latter of which shares with cytochalasin B, its inhibition of cellular motile processes but not that of facilitated glucose transport\(^4\); and ii) millimolar concentrations of the transportable (but not metabolizable) D-glucose analog 3-O-methyl-D-glucose, but not the non-transportable glucose stereoisomer L-glucose. Again, primary...
astrocytes demonstrate similar behavior (Lane et al., data not shown).

**IRON UPTAKE AND ASCORBATE / DHA SHUTTLE**

Cellular uptake of NTBI is well evidence, but less well understood in comparison to the classical transferrine-dependent iron import pathway [9]. Cellular uptake of NTBI may be particularly related to in iron overload diseases such as hereditary hemochromatosis, hypotransferrinemia, and thalassemia, in which plasma iron presents in excess of transferrine-binding capacity [8]. By the analysis of ascorbate-mediated stimulation of NTBI reduction and uptake by human erythroleukemia (K562) cells it is found that DHA loading of cells stimulated both processes (viz. 12-and 2-fold, respectively), yet unlike the reduction of ferricyanide remained inhibitable by extracellular ascorbate oxidase [9]. Furthermore, as cells were able to import iron in a manner inhibitable by cell-impermant ferrous ion chelators, the ascorbate-stimulated iron uptake is clearly dependent on the initial adoption of the ferrous state [8].

This suggests that ascorbate released from cells following uptake and reduction of DHA-mediates direct reduction of ferric to ferrous iron, ferrous iron is then imported (fig. 3). Subsequent addition of DHA to control or loaded cells resulted in a dose-dependent stimulation of both iron reduction and uptake that can be inhibited by cytochalasin B, suggesting response-dependence on DHA uptake via GLUTs. Again, these results are basically reproducible with primary astrocyte cultures [9]. Several possible candidates for the cellular export of ascorbate have been proposed, including exocytosis of ascorbate-containing vesicles, ascorbate-ascorbate homeoexchangers, connexin hemi-channels and volume-sensitive osmolyte and anion channels (VSOACs). VSOAC permeability and ascorbate efflux from cells can be inhibited by generic anion channel inhibitors, such as 4,4'-diisothiocyanato-2,2'-disulfonic acid (DIDS) and 4-acetamido-4'-isothiocyanato-2,2'-disulfonic acid (SITS), suggesting that a significant proportion of ascorbate release occurs via this pathway. It has been observed that DIDS inhibits ascorbate release, ferrireduction and iron uptake to similar degree in K562 cells [4].

**COMPOSITION OF T-PMET**

The reduction of extracellular molecules takes place by outward flow of electrons coming from cytosolic donors, due to the action of tPMET. Enzyme-mediated and/or shuttle-based electron transfer is involved in this trans-plasma membrane flow. (Fig. 5) [10, 11, 12].

Identification of several components has been done in last two decades and characterization at the molecular and biochemical level of some of these components has been done. Among them, some are expressed ubiquitously, some are present in certain cell types, some utilize only a subset of electron donor and acceptor and some are less specific [13].

**A) Electron Donor:** From NADH and NADPH, intercellular reducing equivalents may be derived, catabolic reactions are responsible for production of first co-enzyme Q, where as synthesis of fatty acid and cholesterol takes place by the presence of NADPH/NADP\(^+\) system, these reducing equivalent systems are also required for hydroxylation and detoxification reactions.
Numerous biological functions are affected by the ratios of NAD+/NADPH\(^{14,15}\). To determine the changes in these ratio, several biological techniques such as bio-luminescence, chromatography and cycling assays have been developed. These techniques can determine the change in ratio under both physiological and pathological conditions. As living cells contain enzymes, which are able to hydrolyze pyridine nucleotides, technical problems may arise during their extraction and these techniques also have some limitations, such as concerning sensitivity, reproducibility and interference with other reducing compounds present in assay buffer\(^{16}\).

Intercellular substance like flavonoids and ascorbate (Asc) have been seen to protect cells from extracellular oxidant stressors, in fact they are crucial substrates for tPMET activity in red blood cells, erythrocytes may encounter a verity of oxidants that exert detrimental effects. Abundantly present flavonoids in fruits and vegetables are quercetin and myricetin, which are utilized by erythrocytes and actively promote tPMET activity. Their structure is responsible for their ability to act as electron donor, the B ring structure of catechol is necessary for the reducing activity of these molecules\(^{17}\). Red Blood Cells are dependent on the intercellular Asc level, as is evident from the observation that the treatment of erythrocytes with nitroxide free radical Tempol (2, 2, 6, 6-tetramethyl-4-hydroxypiperidine-N-oxyl) (fig.4) which is responsible for the endogenous Asc depletion (without affecting glutathione or á-tocopherol content)\(^{18}\), where as inhibition of 80% basal rate of ferricyanide reduction were seen in untreated cells\(^{19,20}\). Astrocytes show similar situation, in these cells Asc-dependent tPMET is more important than the NADH-dependent tPMET\(^{21}\). There are two important mechanisms, in which Asc contributes to tPMET, are (i) enzyme-mediated electron transport, in which electron donor is Asc for transmembrane oxidoreductases and (ii) non enzymatic electron transfer, where cells directly release Asc which act as reducing agent thus oxidized to dehydroascorbate (DHA) via intermediate ascorbyl free radical (AFR)\(^{22,23,24}\). DHA further reduced back to Asc by the reducing equivalents coming from cellular metabolite shuttling/cycling mechanism involving other redox couple including superoxidizedioxygen\(^{25,26}\), dihydrolipoic acid/ á-lipoic acid\(^{27,28}\) and reduced glutathione/cysteine\(^{29}\).

**B) Electron Acceptor:** Oxygen is most important extracellular acceptor which fully reduced to water with the generation of reactive oxygen species (ROS) including superoxide (\(O_2^-\)) and hydrogen peroxide (\(H_2O_2\)) which help in the modulation of specific cellular function and signal transduction pathway\(^{30}\). AFR is another physiological substrate which reduced to Asc\(^{24}\) and ferric ion which again reduced to ferrous ion\(^{31}\), ferrous ion is important for the proximal small intestinal epithelium, where enterocytes utilize iron, but before its transportation across the membrane occurs it should be reduced.

**C) Intermediate Electron Carriers:** Intermediate electron acceptors are mainly b cytochromes, flavin and vitamin E, but most widely used electron shuttle is ubiquinone (or coenzyme Q [CoQ]) (Fig.5). It is able to move between membrane bi layer and links the cell from inside to outside.\(^{32}\)

**Fig. 5. Key components of t-PMET**

**D) Enzymes**

1. **NADPH oxidases**

   Superoxide families generating NADPH oxidases also named as Nox or Phox i.e.
phagocytic oxidases. It includes seven proteins (Nox1 to Nox5 and Duox1 and Duox2) and is best characterized class of enzymes present in tPMET. Nox 2 is the first recognized and most extensively studied member, expressed in human phagocytes and responsible for production of superoxide during engulfment of invading microbes\(^\text{[33,34]}\). Members of Nox family are involved in many biological functions such as signal transduction, host defense, development, angiogenesis, blood pressure regulation and biosynthetic processes\(^\text{[35,36]}\). NADPH acts as electron donor for catalyzing the reduction of oxygen to produce superoxide, hydrogen peroxide and oxygen with the help of enzyme superoxide dismutase (SOD). Hydrogen peroxide so formed acts as second messenger molecule. On the basis of structure, the Nox enzymes are classified into three functional groups. They are as follows.

1) Nox 1-4: Nox 2 is the first identified prototype of this family. Cytochrome \(b\)^558, is the catalytic part of Nox 2, it is heterodimer composed of two sub nits, namely, \(p^{22}\text{phox}\) (light chain) and \(gp^{91}\text{phox}\) (heavy chain)\(^\text{[37]}\). Nox2 is usually inactive in resting cells. Nox 1 is expressed in color epithelial cells primarily and also found in vascular smooth muscle cells, uterus and prostate\(^\text{[38]}\). \(p^{22}\text{phox}\) is associated with Nox 1 like Nox 2, Nox 1 requires NoxO1 protein an organizer and NoxA1 protein an activator. Nox 3 mRNA is found in foetal tissues, kidney, liver, lung and spleen \(^\text{[39,40]}\) but mainly present in inner ear like Nox 2 and Nox 1, it also requires \(p^{22}\text{phox}\) but it does not require organizer and activator protein for its enzyme activity. Finally, the expression of Nox 4 is higher in kidney and vascular endothelial cells \(^\text{[11,32]}\). Nox is able to produce a functional diamer with \(p^{22}\text{phox}\), it can produce superoxide anions without intervention of organism and activator protein same like Nox 3.

2). Nox 5: It is found in testis and also expressed itself in T- and B lymphocytes \(^\text{[41]}\). Nox 5 is also related to the other members of the family. Its activity does not require organizer and activator protein, in this way it differs from other Nox isoforms but it requires intercellular calcium concentration and is totally depend on it. In Nox 5 calcium sensitization is archived by two main mechanisms.

a). The first mechanism involves protein kinase C- dependent phosphorylation of Thr494 and Ser 498 present in the FAD binding domain.\(^\text{[42]}\)

b). The second mechanism involves calmodulin binding site present in the NADPH binding domain. \(^\text{[43]}\)

3) Duox 1 and Duox 2: Expression of these oxidases is mainly found in the membrane of thyroid glands \(^\text{[43]}\). They usually produce \(H_{2}O_{2}\) rather than \(O_{2}\). They have the basic structure of \(gp^{91}\text{phox}\) enzymes \(^\text{[44]}\). Therefore superoxide anions are mainly produced which rapidly converted to \(H_{2}O_{2}\) by the help of enzyme dismutase, this process is known as dismutation. They have an additional N-terminal peroxidase like domain present on the outside membrane. These enzymes are calcium responsive enzyme. \(^\text{[45]}\)

### Biological Function

- Nox 1 plays two important roles: immune defense and cell proliferation.
- Nox 2 helps in signaling and also involves in immune defense, it is present in endothelial cells and responsible for endothelial growth factor and thrombin and also implicate in new blood vessel formation. They are also responsible for tumor cell proliferation.
- Most important function of Nox 3 is participation in normal vestibular functions as it is present in the inner ear.
- In kidney Nox 4 helps in oxygen sensing and regulation of erythropoietin synthesis. It also acts as an antimicrobial system as it helps in detoxification of urine wastes by releasing ROS in glomerular filter.
- Main function of Nox 5 is in testis as it promotes oxidative changes which are usually associated with sperm capacitation and acrosome reaction.

### 2. NAD(P)H: quinone oxidoreductase

It is also known as DT-diaphorase or QR1. It is present in cytosole and is homodimeric flavor protein. Its enzyme commission number is 1.6.99.2. Under oxidative condition it is over expressed and trans located to plasma membrane. \(^\text{[46, 47]}\)
Biological Function

- It is a key enzyme for cellular defense against ROS.
- It shows scavenging activity due to the presence of NAD(P)H-dependent superoxidase.
- It is responsible for ubiquinone cycle and facilitates in-out transfer of electron and it also produces redox-labile hydroquinone. Therefore it has a complex metabolic pathway for its protective functions.
- It acts as chemo-protective enzyme, as it is able to reduce quinine-imines, nitro- and azo-compounds.
- It helps in detoxification of xenobiotics and prevents cytotoxic and carcinogenic effects.
- It is able to modulate oncoprotein stability.

3. Disulfide-thiol exchangers: They belong to the family of cell surface proteins and exhibit hydroquinone (NADH) oxidase activity and protein disulfide-thiol interchange activity. They are also known as ENOX proteins as they are located outer side of plasma membrane.

Biological Function: It possesses two important biological functions, first function is to participate in enlargement of cell growth and the second function is that it is the important component of biological clock.

4. Voltage-dependent anion-selective channels: It represents a family of 30-35 kDa integral membrane protein. They are located in outer mitochondrial membrane.

Biological Function: Its major function is to control metabolic trafficking between cytosole and mitochondria by forming pores which are permeable to low molecular weight molecules such as ATP, ADP, succinate and citrate.
- They also help in the release of apoptogenic proteins from mitochondria.

5. Duodenal cytochrome b: Dcytb is also known as Cybrd1, it is the member of cytochrome b561 family. Dcytb mRNA and protein both are induced in response to hypoxia and iron deficiency, they play an important role in iron metabolism.

Biological Function: It plays an important role in uptake of dietary nonheme iron.

6. Cytochrome b₅ reductase: Its enzyme commission number is 1.6.2.2, also known as diaphorase-1 or methemoglobin reductase.

Biological Function

- They are membrane associated enzymes present in all human cells, it helps in fatty acid chain elongation and desaturation, cholesterol synthesis and hydroxylation of xenobiotics such as hydroxylamine and amidoxime compounds. In erythrocytes they help in maintaining hemoglobin in its reduced state.

PATHOLOGICAL ROLES OF ASCORBATE IN T-PMET

Various pathological conditions are regulated by ascorbate in tPMET. These are described below.

Apoptosis

This process is involved in body homeostasis and tissue development. Defect in the process of apoptosis leads to several diseases: Hypotrophy can be caused by excessive apoptosis; insufficient amount of apoptosis can cause cancer due to uncontrolled cell proliferation.

ROS generates due to inhibition of ascorbate in t-PMET which leads to pro-oxidant at plasma membrane and promotes apoptosis.

Cancer

Through mitochondrial oxidative phosphorylation, normal tissues derive their energy by glucose metabolism and produce carbon dioxide and water. Even in the presence of oxygen cancer cells convert glucose into lactose rather than pyruvate. This phenomenon is known as Warburg effect. Ascorbate plays an important role in cancer biology. It may perturb key redox couples which include NAD(P)H/NAD(P)⁺ and CoQH₂/Co Q ratio, it neutralizes free radicals before they can damage DNA and initiate tumor growth and or may act as a pro-oxidant.
helping body's own free radicals to destroy tumors in their early stages [64,65]. The t-PMET is useful for anticancer drug development due to its targeting. Recently Prata et al. reported that in human leukemic cells the primary site of action of new anticancer compound is t-PMET such as [3-(2-chloro-5-methoxy-6-methyl-3-indolymethylene)5-hydroxy-1,3-dihydroindol-2-one,3-{[2,6-dimethylimidazo[2,1-b]thiazol-5-yl]methylene}-5-methoxy-2-indolinone and guanylhydrazone of 2-chloro-6-(2,5-dimethoxy-4-nitrophenyl) imidazo [2, 1-b] thiazole-5-carbaldehyde] having anti-proliferative activity, therefore for the treatment of leukemia, specific targeting of t-PMET may be utilized in combination of ascorbate with standard chemotherapeutic drugs. Treatment with ascorbyl stearate resulted in concentration-dependent inhibition of cell proliferation cancer cells [66, 67]. The anti-proliferative effect was found to be due to the arrest of cells in S/G2-M phase of cell cycle, with increased fraction of apoptotic cells. Considerable biochemical and physiological evidence suggests that ascorbic acid functions as a free radical scavenger and inhibits the formation of potentially carcinogenic N-nitroso compounds from nitrates, nitrite in stomach and thus offers protection against cancer [68-69].

Cardiovascular diseases

T-PMET regulates cardiovascular diseases by controlling the redox state and so, the redox-dependent signaling pathways in endothelial cells. It has been proved that Hyperhomocysteinemia stimulates ferricyanide reductase activity and cytochrome b5 reductase expression, thus forming a potential link between t-PMET, oxidative stress, and endothelial dysfunction [70]; Jessup’s et al. found that enhanced t-PMET activity induces low-density lipoprotein oxidation [71], thus up regulation of t-PMET may be numbered among atherogenic factors. Nox1, Nox2 and Nox4 have been shown to be implicated in pathways leading to steatosis and insulin resistance in the liver, as well as to pancreatic B-cell dysfunction, thereby allowing progression from the metabolic syndrome to type 2 diabetes [76].

CONCLUSIONS

Cell plasma membranes have complex signaling systems for regulating cellular metabolism. Several research studies basically concentrate on enzymatic tPMET systems; however several examples of ‘shuttle-based’ tPMET systems have been documented as well, including ascorbate/DHA, dihydrolipoic acid/alpha-lipoic acid, reduced glutathione/cysteine and superoxide di oxygen shuttles. t-PMET unregulated optimal NAD+ level which is required for the production of ATP in glycolysis under low mitochondrial activity whereas increased activity
is responsible for superoxide anion and hydrogen per oxide production which enhance cell growth by regulating signaling molecule. As with classical enzyme-mediated tPMET systems, these 'shuttle-based' systems result in the net transfer of metabolically derived reducing equivalents from the cytoplasmic compartment to the extracellular space. In the extracellular space, the fate of these reducing equivalents depends on the particular redox couple involved. Many enzymes present in t-PMET have inducible systems, which are activated by variety of extracellular effectors such as growth factors, cytokines and hormones, so that oscillation of ROS production serves the need of different tissues. The example of shuttle-based tPMET is transplasma membrane ascorbate/DHA cycling, which leads to redox change linked to cell metabolism. Transplasma membrane ascorbate DHA cycling may contribute significantly to NTBI ferric reduction prior to ferrous uptake. As an example, superoxide production at cell surface may not be derived from any of NOX isoform action, non -mitochondrial oxygen consumption was inhibited by extracellular NADH in several glycolytic cancer cell lines whereas the oxygen burst observed in activated platelets and leukocytes was demonstrated to be stimulated by the presence of exogenous NADH From the involvement of ascorbic acid present in t-PMET in several pathological conditions, it is mandatory to understand, in the future, the relative contribution of each oxidase system to ROS generation and shuttle-based t-PMET systems; this will help us to design novel therapeutic approaches. Thus, though ascorbic acid was discovered in 17th century and Trans- Plasma membrane electron transport (t-PMET) has been established in the year 1960s, their role is important in human health and disease, still remains a mystery.

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