Relation between Mitochondrial Thiols and Thioredoxin System in Some Cancers

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Research Article

Abstract: Mitochondria are the site for generation of highly reactive oxygen and nitrogen species. These bring about oxidation and nitration of various biomolecules in the vicinity thus affecting their functions. Mitochondrial thiols involved in many important functions may thus be affected. Thioredoxin and Thioredoxin reductase, function to restore modified proteins. Many pathological conditions like cancer are associated with increased production of reactive species generating oxidative stress. The prevalence of breast cancer and that of prostrate and oral cancer including cheek and tongue is increasing. **Objectives:** The present study was carried out to find out levels of mitochondrial total and membrane protein thiols. Plasma NOx, nitrothiol and nitrotyrosine levels were measured to know the role of NO• and Thioredoxin and Thioredoxin reductase levels were measured as antioxidant defense proteins. **Material and methods**: Mitochondria were isolated from tissues of patients of breast, prostate, cheek and tongue cancer and non-malignant tissues. Mitochondria were lysed and lysate was used for estimation of Trx, TR, nitrothiols, total and membrane thiol concentrations. The cell lysate was used for estimation of nitrotyrosine. NOx levels were estimated in plasma samples. **Observation**: It was observed that levels of plasma NOx, were not altered significantly and nitrothiol and nitrotyrosine were not detected. However levels of mitochondrial total and membrane protein thiols were significantly decreased ($p<0.05$).While levels of Thioredoxin and Thioredoxin reductase were increased significantly ($p<0.05$). **Conclusion**: It can be concluded that decrease in mitochondrial thiols causes increase in Thioredoxin and Thioredoxin reductase in cancers under present study which may be independent of NOx, nitrothiol and nitrotyrosine.

Key words: Nitric oxide, nitrothiol, nitrotyrosine, Thioredoxin, Thioredoxin reductase

Introduction

Mitochondrial thiols are involved in various functions like electron transport, oxidative phosphorylation, membrane transport, cell apoptosis. These functions are affected when thiol status is altered. Mitochondria are able to generate reactive oxygen species (ROS) and reactive nitrogen species (RNS), thus favouring conditions of potential oxidative stress. This results in modification of mitochondrial thiols[(1)]. Nitric oxide (NO°) is free radical and major contributor of RNS generation. NO° is synthesized from L-Arginine in the reaction catalysed by nitric oxide synthase. It is oxidizing as well as nitrating agent which can traverse biological membranes. At equimolar concentration, NO° reacts with superoxide the major ROS to form peroxynitrite[(2)]. Peroxynitrite is responsible for nitration of free and protein bound tyrosine residue forming nitrotyrosine; footprint of increased NO° production. In addition to this NO° can react rapidly in the intracellular environment to form nitrite and nitrate, S-nitroso-thiols etc [(3)]. Proteins thus oxidised are reduced back by various systems in the body. Thioredoxin system is one of them. It consists of Thioredoxin reductase (TR), Thioredoxin (Trx) and NADPH. Trx in its active form (reduced) provides reducing equivalents to target molecules like peroxiredoxins (cellular antioxidants) ribonucleotide reductase (involved in DNA synthesis), and various transcription factors. During these reactions Trx gets oxidised. This oxidised Trx is reduced back to its active form in presence of TR. Constitutive Trx and TR expression has been observed in several cell types of the mammalian body, including keratinocytes of the skin, placental cells, liver cells, secretary cells, and leukocytes [(4, 5)] Physiological stimuli, including UV light, hydrogen peroxide and mitogens can induce the expression of Trx and TR pointing at an important role in protection against oxidative stress and in regulating cell growth and cell death [(6)]. Many pathological conditions are associated with increased oxidative stress, cancer is one of them. Among the cancers, prevalence of breast cancers in females and cancers of prostate and oral cancers including cheek and tongue is increasing in men. Keeping these facts in mind present work was designed to know whether mitochondrial thiols are affected in cancers of breast, prostate, cheek and tongue. Secondly to know whether NO° and RNS like nitrotyrosine, nitrothiols are generated and probable role of these in modifying mitochondrial thiols. The third aim is to know the levels of TR and Trx the antioxidant defense proteins.

Material and Method

Present study was carried out after institutional ethical approval and prior consent. Cancer tissue samples were collected from operation theatre of surgery department. The fibrous tissue and blood vessels were removed. Tissue samples were homogenized centrifuged at 2000g for 10 min at 2^0 C in cooling centrifuge. Cell debris was removed. The supernatant was used to estimate protein [7] and nitrotyrosine [8] concentration. Remaining supernatant was spun at 10000 g for 15 min in cooling centrifuge to get mitochondrial pellet. The pellet was washed 3-4 times. Mitochondria were thus isolated[9] from 37 tissues of breast cancer, 32 tissues of prostate cancer, 42 tissues of cheek cancer, 35 tissues of tongue cancer and 38 non-malignant tissues. Mitochondria were lysed by freezing and thawing. The lysate was used for estimation of protein, nitrothiols [10], Trx [11], TR [12], and total thiol concentrations [13]. The membrane protein thiols were estimated [14]. NOx (nitrate plus nitrite) levels were estimated in plasma samples [15, 16]. The samples were run in duplicate. For each sample; the mean of the two values was taken. The statistical significance was calculated by Mann –Whitney U test by using NCSS-PASS statistical software. Statistical significance was chosen as $p<0.005$.

Table 1

	Plasma NOx (µmol/L) Mean ± SD	Total thiol level(nmol/mg of protein) Mean ± SD	Membrane protein thiol level(nmol/mg of protein) Mean ± SD	Trx level (pmol/mg of protein) Mean ± SD	TR level (pmol/mg of protein) Mean ± SD
Controls (n=38)	35.61±5.9	111.35±11.6	56.35±5.9	367.5±103.02	61.66±5.9
Breast cancer (n=37)	34.6±6.15	68.67*±8.66	35.95*±8.74	3027.18*±904.86	540.87*±8.74
Prostate cancer (n=32)	34.04±5.5	69.97*±14.9	36.83*±10.66	2439.11*±455	625.88*±10.7
Cheek cancer (n=42)	35.63±7.6	65.65*±10.7	34.16*±8.4	2568.98*±851.1	603.26*±32.2
Tongue cancer (n=35)	34.09±6.3	76.82*±8.2	38.32*±5.3	1466.26*±164.0	188.05*±5.3

*= $p<0.05$ significantly different from control group at $p<0.05$. (Nitrothiol and nitrotyrosine were not detected in control and experimental group)

Observations and Results

The table 1 shows the levels of plasma NOx, mitochondrial total and membrane protein thiols and the levels of Thioredoxin and Thioredoxin reductase in controls as well as experimental group.

Discussion

Mitochondria are major site for generation of ROS and RNS. RNS include highly reactive NO°, nitrothiols and nitrotyrosine .These reactive species react with biomolecules in the vicinity. The thiol containing mitochondrial proteins are one of the target sites. In the present study the levels of mitochondrial total and membrane protein thiols were found to be decreased significantly ($p<0.05$) in cancer group as compared to control group. This decrease could be due to oxidative and/or nitrative modification of thiol groups. To know whether NO° has any role in the process, plasma NOx levels were determined. When the levels of NOx were compared with controls, no significance (p=NS) was observed. Reactive species of NO° like nitrotyrosine and nitrothiol were also not detected in different cancers under study. This may be because of various reasons, such as inadequate supply of arginine or cofactor tetrahydrobiopterin resulting in an "uncoupling" of NOS from the production of NO°. As a result of this superoxide anions are produced instead of NO° generating oxidative stress [17]. Another major mechanism of decreased production of NO° may be due to an increase in the arginase activity which decreases arginine availability for NO° production and increases production of ornithine for polyamines synthesis [18]. Polyamines during catabolism are known to produce H_2O_2. This H_2O_2 and superoxide anions produced during uncoupling of NO° may be major contributory factor for decreased levels of mitochondrial total and membrane protein thiols, when NO° and RNS are not playing major role in modifying thiols. The levels of antioxidant defense proteins Trx and TR are increased significantly ($p<0.05$).The increase might be to restore the function of modified thiol groups and are upregulated in certain tumours, to protect cancer cells from oxidative stress [19] and express high levels of antioxidant proteins[20]. Mitochondrial thioredoxin system is a potential source of disulfide reductase activity required for maintaining mitochondrial proteins in their reduced state [21]. However the levels of mitochondrial total thiols and membrane protein thiols were found to be decreased in presence of elevated levels of Trx and TR as the effects of the two

are no longer solely beneficial for the patient once the tumour is established [22]. The increased levels of the Trx and TR as observed in the present study might be playing role in tumour progression by various mechanisms [23, 24]. It can be concluded from the present study, that reduction in mitochondrial thiol in cancers of breast, prostate, and oral like cheek and tongue are independent of NO°, nitrothiol and nitrotyrosine. Antioxidant defense system responds to it by elevating the activity of TR and Trx. However elevated levels of these two proteins might not play role in restoring mitochondrial thiols.

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