A COMPARATIVE STUDY OF REACTIVE OXYGEN SPECIES (ROS) RELATED PARAMETERS IN RAT TISSUES

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ABSTRACT

Reactive oxygen species (ROS) are believed to be responsible for pathogenesis of various diseases affecting tissues and systems. ROS generated by mitochondrial electron transport chain as well as extra-mitochondrially are eliminated by the respective defense mechanisms. We checked the activity of ROS generating system such as xanthine oxidase and also the parameter of ROS defense mechanism e.g. superoxide dismutase (SOD), catalase, glutathione peroxidase (GPox), reduced glutathione content (GSH) and glucose-6-phosphate dehydrogenase (G6PDH) in mitochondrial and post-mitochondrial fractions from various tissues (liver, kidney, brain and heart) of normal rats. Extent of lipid peroxidation (LPO) which is immediate consequence of ROS generation was also examined. Our results shows that significant tissue-specific differences exist in mitochondrial and cytosolic ROS generating systems and ROS defense mechanisms.

INTRODUCTION

The metabolic activities differ considerably from tissue to tissue. Because of this the energy requirements of the tissues also show considerable variations (1-6). Mitochondria are the major source for meeting the energy requirements of any given tissue and their ability to synthesize ATP is directly related to the rates of substrate oxidation (1-6). An inherent problem during oxygen utilization in the mitochondrial electron transport chain is the oxygen slippage which is estimated to be from 3-5% (7-9). The oxygen slippage results in the generation of the superoxide radical (O_2^-) which in turn can lead to generation of other reactive oxygen species (ROS) (8-11). O_2^- and ROS can also originate from extra-mitochondrial systems such as microsomal electron transport chain and other enzymes such as xanthine oxidase, aldehyde oxidase, dihydroorotic dehydrogenase, and a group of flavoprotein dehydrogenases (7,12,13). O_2^- is also produced by intact granulocytes during phagocytosis (7, 12, 14). The oxygen consumption rates and metabolic activities of the tissues differ considerably (1-6). Hence it may be anticipated that the quantum of oxygen slippage and ROS generation from mitochondria and cytosolic systems can show tissue-

specific differences. This in turn may be reflected in terms of the mitochondrial and cytosolic defense mechanisms involved in the elimination of O_2^- and ROS (7, 9).

The ROS are implicated in etiology of several diseases affecting tissues and systems (8, 14-21). Hence it is of interest to know about the similarities and differences in the ROS related systems in different tissues. This information can have bearing on role of ROS in pathogenesis and etiology of diseases (8, 14-21). With this objective in mind we examined the ROS metabolism related systems in tissues such as liver, kidney, brain, and heart from control male rats. Results for the latter three tissues were compared with liver since liver is the major metabolic tissue.

MATERIALS AND METHODS

Bovine serum albumin fraction V (BSA) and 5, 5' - dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from SRL, India. Sodium salts of NADP⁺, glucose-6-phosphate (G6P) and 4-morpholinopropanesulfonic acid (MOPS) were obtained from Sigma, USA. Xanthine was purchased from Hofmann La Roche, Switzerland. Reduced glutathione (GSH) was purchased from Hi Media, India. All other chemicals were of the highest purity grade and were purchased locally.

Isolation of mitochondria and post-mitochondrial fractions

Adult male albino rats of Charles-Foster strain (8-10 week old) weighing between 250-300 g were used in the present studies. The animals were killed by

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decapitation and the tissues i.e. liver, kidneys, brain and heart were quickly removed and placed in beakers containing chilled (0-4°C) isolation media. The isolation medium for liver, kidneys and brain contained: 0.25 M sucrose, 10 mM Tris-HCl buffer, pH 7.4, 1 mM EDTA and 250 g BSA/ml. The isolation medium for heart contained: 0.3 M sucrose, 5 mM MOPS, pH 7.4, 1 mM EDTA and 250 g BSA/ml.

The tissues were minced if necessary and washed repeatedly with the isolation medium to remove adhering blood and 10% (w/v) homogenates were prepared using a Potter-Elvehjem type glass-Teflon homogenizer. The nuclei and cell debris were sedimented by centrifugation at 650 x g for 10 min and discarded. The supernatant was subjected to a further centrifugation at 7,500 x g for 10 min. The resulting mitochondrial pellet was washed by suspending gently in the isolation medium and by resedimenting at 7,500 x g for 10 min. Finally, the mitochondria were suspended in the isolation medium to give about 12-15 mg mitochondrial protein/ml. The post-mitochondrial supernatant was then centrifuged at 12,000 x g for 10 min to remove light mitochondria (22). The pellet was discarded and the resulting supernatant was used as the source of cytosolic enzymes. Simultaneously, the volume of the supernatant was recorded.

Measurements were carried out to determine the extent of lipid peroxidation (LPO) (23), glutathione (GSH) contents (22) and xanthine oxidase (XO) (13, 24), superoxide dismutase (SOD) (25, 26), catalase (13, 27), glutathione peroxidase (GPox) (28) and glucose-6-phosphate dehydrogenase (G6PDH) activities (29).

Protein estimation was according to the method of Lowry *et al.* using bovine serum albumin as the standard (30).

Statistical evaluation of the data was done by Students' t-test.

For a given enzyme, the total mitochondrial activity/g tissue was computed based on the mitochondrial protein content in the various tissues which have been reported to be: 50.6, 64.0, 33.5 and 65.0 mg mitochondrial protein/g tissue, respectively for liver, kidney, brain and heart (31, 32). Total cytosolic activity/g tissue was computed based on the volume of the post-mitochondrial supernatant which was used as the source of the enzyme.

RESULT AND DISCUSSION

The extent of lipid peroxidation (LPO) measured as thiobarbituric acid reactive species (TBARS)/mg protein in the mitochondrial and the post-mitochondrial fractions from the four tissues is shown in Fig. 1. Thus,

compared to values for the liver mitochondria, the LPO content in kidney and brain mitochondria was 1.7 and 1.5 fold higher while that in heart was the highest i.e. 3 fold higher. The specific LPO content in the post-mitochondrial fraction from liver and kidney was comparable while that in the other two tissues was about 1.7 and 2.4 fold high. Thus the contents of LPO were highest in mitochondrial and post-mitochondrial fractions from the heart tissue.

The XO activity was highest in the liver followed by the kidneys; compared to the liver, the activities in the brain and heart were only about 16 and 0.5 %. The enzyme XO generates the O_2^- as a reaction intermediate (7, 12).

The O_2^- thus generated either mitochondrially or in the cytosol is dismutated to H_2O_2 by Mn SOD in mitochondria and Cu SOD in cytosol, respectively (12, 33). As can be noted (Fig. 1) the mitochondrial SOD activity was the highest in the liver; the activities in the other tissues were comparable and were about half of that noted for liver mitochondria. The cytosolic SOD activity was, once again, highest in the liver and interestingly, the activity was about double of that in the mitochondria. Compared to the liver, these values for the other three tissues ranged from 13 to 23%. Thus Cu-Zn SOD seems to be the predominant form in the liver whereas Mn-SOD is the predominant form in the other tissues.

The H_2O_2 produced by SODs can be eliminated by several ways i.e. either by the action of catalase (13, 27) or by glutathione-dependent routes involving selenium-dependent or selenium-independent glutathione peroxidases (GPox) (28).

It is of interest to note that once again the catalase activity is the highest in the liver followed by kidneys whereas the brain and the heart tissues possess very low catalase activity amounting to about 10% compared to the liver value. It may also be pointed out here that relative abundance of catalase compared to the mitochondrial or post mitochondrial SOD was the highest in the kidneys followed by the brain and the liver; these values were the lowest in the heart. The specific activity of mitochondrial GPox (Fig. 1) was the highest and comparable in the liver and the kidneys; these values for brain and heart were about half. Cytosolic GPox activity in the post-mitochondrial fraction was relatively higher than the mitochondrial enzyme in the liver and values were almost comparable for liver, brain and heart. Interestingly, this activity was about 2 times higher in the kidneys.

Finally, the specific GSH content in mitochondria was found to be comparable for liver and kidneys but the contents were relatively higher in brain and heart (20 to 40% higher compared to liver or kidney). The GSH content in the post-mitochondrial fraction was relatively

Fig. 1. ROS parameters in mitochondrial and post-mitochondrial fractions from rat liver , kidney , brain and heart .

Results are given as mean $\pm$ SEM of 8-16 independent experiments.

a, $p < 0.001$ compared with the corresponding values of liver.

much higher in all the tissues compared to their respective mitochondrial values. Thus, the cytosolic GSH content was highest in the liver followed by heart whereas the kidneys showed the lowest values (Fig. 1).

Regeneration of GSH requires NADPH as the electron donor. Amongst other enzyme systems, the enzyme G6PDH plays a significant role in generation of the reducing equivalent in the form of NADPH (7). It can be noted that the G6PDH activity was highest in the liver and the lowest in the kidneys; the other two tissues showed intermediate values (Fig. 1).

While the specific activities and/or contents of a given parameter give useful information, the potential of the tissue can be realized in terms of the total content per unit weight of the tissue. These values expressed in

units/g tissue are shown in Fig. 2. Thus the total content of LPO was highest in the heart mitochondria. This would agree with the reported high respiration rates in the heart mitochondria (5). The LPO content in the post-mitochondrial fraction was the highest in the liver which would correlate well with the high metabolic/synthetic activity and the strong P_{450} system in the tissue. Consistent with this assumption is the finding that the XO activity was highest in the liver as reported by earlier workers (34). Taken together, the results would suggest that extra-mitochondrial generation of ROS is significantly high in the liver than in the other three tissues examined (Fig. 1 and 2). Also, liver seems to be the major site of purine catabolism, followed by kidneys. The observation that the Cu-Zn SOD content was higher than the Mn-SOD content in the liver would also substantiate the

Fig. 2. Tissue Content of ROS parameters in mitochondrial and post-mitochondrial fractions from rat liver , kidney , brain and heart .

Content is given per gram tissue. Results are given as mean $\pm$ SEM of 8-16 independent experiments.

a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.002$ and d, $p < 0.001$ compared with the corresponding values of liver.

assumption that in the liver magnitude of ROS generated extra-mitochondrially may be higher. The reverse seems to be the case for the other tissues (Fig. 2). The fact that the GPox contents were higher than catalase would suggest that glutathione mediated route may constitute a strong protective mechanism even if H_2O_2 escapes action of catalase. This is especially true for the GPox content in the post-mitochondrial fractions from all the tissues. Consistent with this assumption are the results which show that the GSH content in the post-mitochondrial fractions from all the tissues was generally very high (Fig. 2). Interestingly, the G6PDH activity was the highest in liver compared to the other tissues. It is possible that either the requirement of GSH in tissues other than liver is low or that these tissues may use alternate mechanisms such as isocitrate dehydrogenase, malic

enzyme and/or mitochondrial energy-linked nicotinamide nucleotide transhydrogenase (35-37) for generating NADPH which is ultimately used towards regeneration of GSH. However, these possibilities need to be explored experimentally.

In conclusion, results of our present studies have shown that the ROS related systems exhibits tissue-specific differences.

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