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RESEARCH ARTICLE

THE INCREASED OF CARBONIC ANHYDRASE IN LIVER TISSUE OF RAT INDUCED BY CHRONIC SYSTEMIC HYPOXIA

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ABSTRACT

Background: Carbonic anhydrases (CAs) are metalloenzymes which catalyze the reversible hydration/dehydration reaction of CO2, in order to maintain the cell homeostasis. These enzymes are found in various tissues and involve in a number of different physiological processes, including ion transport, acid-base balance, bone formation, gluconeogenesis and so on.

Objective: To examine the specific activity of CA and to observe the liver tissue respond to oxidative stress by measured the malondialdehyde (MDA) concentration, in rat liver tissue induced by chronic systemic hypoxia for 1, 3, 5, 7 and 14 days of hypoxia.

Results: The study showed that the activity of CA which induced by chronic systemic hypoxia significantly increasing at early exposure to the hypoxic condition, at day 1 and days 3 of hypoxia (0.281 and 0.262 nmol/mg protein/minute compared to control 0.155 nmol/mg protein/minute) ($p<0.05$). No statistically difference at treatments of hypoxia 5, 7 and 14 days. The concentration of MDA also increased significantly in day 3 of liver tissue hypoxia (0.013 nmol/mg compared to control 0.009 nmol/mg liver tissue) ($p<0.05$), and no statistically differences at day 1, 5, 7, and 14 days of hypoxia.

Conclusion : There was damage of membrane cells affected by oxidative stress in liver tissue of rat induced by chronic systemic hypoxia.

Keywords : Carbonic anhydrase, MDA, Liver

INTRODUCTION

Hypoxia is a condition where cells undergo insufficiency of oxygen supply. Hypoxia has been long considered as a major cause of the failure of radiotherapy, heart failure, and up-regulation of CA, a transmembrane enzyme found to be overexpressed in various tumors and associated with tumor hypoxia, which now becomes targeting for cancer treatment.[1,2] Recent studies have shown the importance of pH in cell death under hypoxia, thus mechanism of pH regulation is likely to be a vital pathway to survival.[3]

One of the important and fundamental functions of CA is maintenance of a pH at around 7.4[4] through reversible hydration of CO2. A remarkable number of physiological and biochemical processes depend upon this reaction. Several latest research in our Department shows that rats induced by chronic systemic hypoxia related to oxidative stress and increased of HIF-1α in liver tissue,[5] induce lesions formation in gastric mucosa,[6] and increased plasma B-type natriuretic peptide-45 (BNP-45) in the ventricular myocardial.[7]

The presence of these ubiquitous enzymes in so many tissues and in so different isoform represents an attractive goal for the design of inhibitors with biomedical applications. CA inhibitors are clinically used as antiglaucoma drugs, antitumor agents, antiobesity agents, and a marker of tumor hypoxia.[3,8] Therefore, CA is an interesting enzyme to study in relationship to hypoxia.

In this work, the activity of CA enzyme in an important rat organ, a liver under hypoxia condition was evaluated by Ozdemir method. MDA was measured in tissue by Wills method.

MATERIAL AND METHODS

The following chemicals were used in the study: p-nitrophenyl acetate was obtained from Sigma Chem. Co; tetraethoxypropane solution, trichloro-acetic acid solution (TCA) and thiobarbituric acid solution (TBA), Tris-SO4 buffer, phosphate buffer saline, and dithiobisnitrobenzene (DTNB) from E. Merck.

Experimental Animals and Protocol

Five groups of 6-8-wk-old Sprague Dawley rats (n = 6), weighing 150-200 g at the time of recruitment, were kept in the animal house of Department Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia. The control group was housed and kept in normoxic, while the hypoxic groups were exposed to the normobaric-hypoxic stimulus ((10% O2: 90% N2) for 1, 3, 5, 7 and 14 days, in the plexiglass hypoxic chambers.

The oxygen tension inside the chambers was continuously monitored by an oxygen meter (OX-12B, MEIE Shanghai, PRC). All the animals were maintained on 12h:12h light-dark cycle and supplied with food and water ad libitum. At the end of each treatment, the animals were euthanazied by decapitation and the was taken out. The tissue was weighed, divided into aliquot and frozen at -86oC. The body weight was measured before and after induced with hypoxia condition.

All the procedures were approved by the Ethical Committee of Medical Faculty Universitas Indonesia No.431/PT02.FK/ETIK/2010.

Determination of Carbonic anhydrase (CA) activity

The total activity of CA was performed by Osdemir method with a

slight modification.[9] The assay system consisted of 50 µL of liver homogenate containing 700 µL of 0.05 M Tris-SO4 buffer (pH 7.4) and 750 µL of 3 mM p-nitrophenyl acetate (pNPA). The change in absorbance at λ 348 nm was measured over a period of 3 minutes before and after adding hemolysate. One unit of enzyme was expressed as 1 µmol of released p-nitrophenol per minute at room temperature.

Determination of MDA in liver tissue

MDA measurement of the samples was determined by the method of Wills[10] using MDA standard to the appropriate concentration range from 0.0375 – 3.75 nmol/L.

Freshly 100 mg samples were prepared using 1 ml of phosphate buffer saline (0.2 M, pH 7.4) and homogenized with a tissue homogenizer (Potter-Elvenjehm), followed by centrifugation 5000 rpm for 5 minutes. Each supernatant was collected and used for the MDA measurements. One mL of thiobarbituric acid was added to the 200 µL supernatant and boiled for 10 minutes in a water bath. Absorbance was measured at λ 530 nm. The concentration of MDA was calculated using a series of a standard curve.

Protein determination

Protein of the samples was determined by the method of Lowry et al[11] using bovine serum albumin (BSA) as the standard to the appropriate concentration range from 0.1 – 0.8 mg/mL. The samples were measured by spectrophotometry at λ 280 nm.

Statistical analysis and data presentation

All data are presented as mean ± SD. The significant of the mean differences between the hypoxia and control groups was assessed by one-way analysis of variants (ANOVA). Means were considered significantly different at level $p<0.05$.

RESULTS

The differences of the body weight shown that in the control group of rat, the average body weight increased about 70 mg along with the experiment. In contrary, in the treatments groups of hypoxia, the body weight was decreased significantly about 32-48% with the length of exposure to hypoxia $p<0.05$) compare to control group.

Determination of Carbonic anhydrase (CA) activity in liver tissue

Total CA of homogenates of liver hypoxic groups and control group are shown in figure 1 as Mean ±SD.

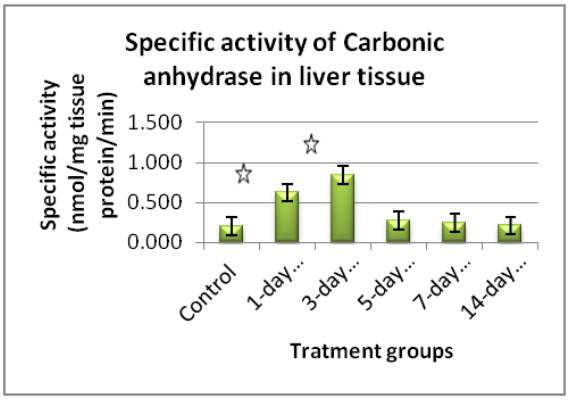

Figure 1. The specific activity of Carbonic anhydrase in liver tissue homogenate ($p<0.05$)

Total CA activities in liver statistically significant differences were found between the control and hypoxic groups in 1 day of hypoxia and 3 days of hypoxia ($p<0.05$). The total activity of CA significantly higher in 3 days of hypoxia (0.843 compared to 0.199 nmol/mg protein/minute), and the activities decreased sharply at day 5, 7 and the end of treatment at day 14. There were no statistically significant differences between

group 5, 7 and 14 days of hypoxia compared to control group ($p>0.05$).

Determination of MDA in liver tissue

The concentration of MDA in liver tissue shown in figure 2. It is shown that MDA concentration in hypoxic groups was significantly increased in day 3 of hypoxia (0.0132 nmol/mg liver tissue compared to control 0.0089 nmol/mg liver tissue) ($p<0.05$). There were no significant differences in MDA concentrations between day 1, 5, 7 and 14 days of hypoxia compared to control group ($p>0.05$)

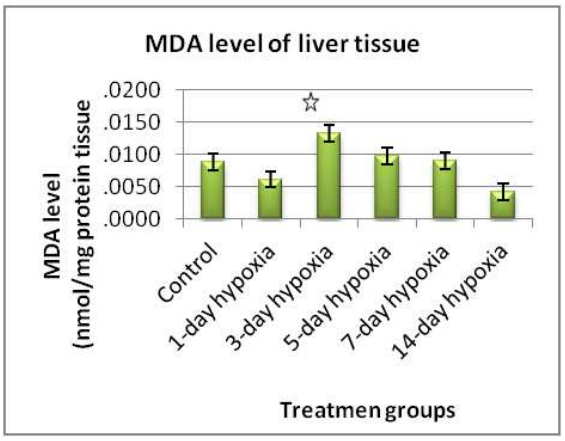

Figure 2. The MDA level of liver homogenate ($p<0.05$)

DISCUSSION

A fundamental paradigm of whole-body acid/base regulation is the maintenance of favorable extracellular pH at around 7.4. The major source of acid in cells is aerobic and anaerobic cellular respiration which generates CO2 and lactic acid respectively. If it allowed accumulating inside cells, intracellular pH would fall to dangerously low levels which affect cell function, growth, and division. One of the mechanisms of the cells to regulate the acid/base homeostasis is through the activity of the carbonic anhydrase enzyme which catalyzes the reversible hydration/dehydration reaction of CO2, producing equivalent H+ and HCO3-.

This condition showed that in the early exposed to hypoxia condition (day 1-3), the activity of liver CA increased significantly in order to maintain the homeostasis of acid/base balance in the cells. In Hypoxia condition, the cellular metabolism changed from aerobic to anaerobic conditions. Anaerobic cellular respiration produces lactic acid and leads to acidosis. One of the metabolic reactions to override it is to induce gluconeogenesis in order to maintain the ATP production of the cells. The pH of the cells, besides multiple membrane transport mechanism to extrude acid into the extracellular environment and maintain a favorable pH intracellular of ~7.2, activity of CA also induced.[3,4] The activity of CA were decreased in day 5, 7 and 14 days of hypoxia, probably because of the capacity of the liver cells to maintained stress of hypoxia were decreased because of the decreasing of the whole body defense, it showed by the decreasing of body weight to about 40% in day 5-14 of hypoxia. The other plausible explanation is because liver CAs is a membrane-bound enzyme which can damage because of radical oxygen species (ROS) which increase in hypoxic condition.[2,13]

We measured the production of MDA as a product of lipid peroxidation, to examine the role of oxidative stress in rat's liver tissue exposed by chronic systemic hypoxia. Hypoxia is a condition of lack of oxygen in the cell, can induce the liver injury. A lack of oxygen in the cell will affect the electron transport chain in mitochondria, In this study, we found that the MDA concentration was increased significantly at hypoxia-induced for 3 days, but no statistical differences in day 1, 5, 7, and 14 days of hypoxia. It's probably

because of the ROS formation was increased larger in the early of rat exposed to hypoxia (day 3) then the later. It mentions that susceptibility to hypoxic condition depends on the type of organism and tissue.

CONCLUSION

The specific activity of carbonic anhydrase of rat's liver tissue induced by chronic systemic hypoxia was increased in the early exposed to hypoxia condition as respond to the lack of oxygen in tissue. The oxidative stress seemed too increased in early exposure to hypoxia and no significant difference between 5, 7, and 14 days of hypoxia.

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