THE RESPONSE OF LIVER LIPID PEROXIDATIVE AND ANTIOXIDANT DEFENSE SYSTEMS OF PROTEIN UNDERNOURISHED RATS TO LIVER REGENERATION

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ABSTRACT

The response of liver lipid peroxidative and antioxidant defense system of protein undernourished rats to liver regeneration induced by partial hepatectomy was examined in rats. Animals were divided into four groups; A,B,C and D of four animals each. Animals in group A were maintained on 16% casein diet while those in groups B, C and D were placed on low-protein diet (5% casein) for fourteen weeks and fed ad libitum. 72 hours before sacrifice, partial hepatectomy was carried out on animals in group D while animals in group C were sham-operated. The results show that protein undernutrition induced an increase in lipid peroxidation but reduced catalase activity, glutathione level and superoxide dismutase activity when compared with well-nourished rats. Liver regeneration however, resulted in significant increases in lipid peroxidation and catalase activity but significant reductions in glutathione level and superoxide dismutase activity in protein undernutrition rats when compared with their sham-operated counterparts. These results suggest that liver regeneration induced by partial hepatectomy exacerbates lipid peroxidation in protein undernutrition rats and that Catalase plays a major role in the mopping up of reactive oxygen species generated following liver regeneration in partially hepatectomised protein undernutrition rats.

KEY WORDS

Lipid peroxidation, Antioxidants, Protein-undernutrition, Liver regeneration, Partial hepatectomy.

INTRODUCTION

Protein undernutrition (PU) is a major problem in the Tropics and one of the abnormalities found in kwashiorkor is reduced concentration of antioxidants, suggesting a compromised capacity to neutralize free radicals, which are known to induce tissue damage (1). Biochemical signs of markedly increased lipid peroxidation with decreased antioxidants have been observed in the liver (2) and plasma of Protein-undernourished rats. Lipid peroxidation (LP) is one of the cellular pathways involved in oxidative damage. Oxidative stress, which is an excessive production of reactive oxygen species (ROS), can damage cells by triggering LP and by altering protein and nucleic acid structures (3, 4).

The liver has a remarkable capacity to regenerate after injury. Within a week after partial hepatectomy (PH), which, in typical experimental settings entails surgical removal of two-third of the liver, hepatic mass is back essentially to what it was prior to surgery. PH leads to proliferation of cells within the liver, including hepatocytes, biliary epithelial cells and endothelial cells (5). Reports on the role of liver regeneration induced by PH on the liver lipid peroxidative system of well-fed rats have been controversial. While some reported increased lipid peroxidation (6) others have reported reduced LP (7,8) following PH in well-fed rats. However, there are no reports on the effect of liver regeneration on protein undernourished subjects and so, it is not known whether PU could influence the response to liver regeneration in anyway.

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The present report is an attempt to investigate the effect of liver regeneration on liver lipid peroxidative and antioxidant defense systems of protein undernourished (PU) rats.

MATERIALS AND METHODS

All reagents, chemicals and casein used were obtained from Sigma chemical co; U.S.A. and BDH chemicals Ltd. Poole, England.

Sixteen 5 weeks old male wistar rats were purchased from the Faculty of Natural Sciences, Federal University of Agriculture, Abeokuta, Nigeria, for the purpose of this experiment. The animals were housed in wooden but adequately-ventilated cages and were divided into four groups; A,B,C and D with four rats to each group. Animals in group A were placed on normal diet (16% casein) while those in groups B, C and D were placed on low-protein diet (5% casein) for fourteen weeks and both food and water were supplied *ad libitum*. Normal and low-protein diets were prepared according to Olowookere (9). Two-third partial hepatectomy was carried out on each of the animals in group D, 72 hours before sacrifice (5, 10). Animals in group C were each sham operated, 72 hours before sacrifice to serve as controls for the partially hepatectomised groups. Food and water were supplied during the 72 hours period. The animals were sacrificed after anesthesia with diethyl ether. The liver samples were collected for analysis. All experimental protocols were conducted following international guidelines on Animal Ethics.

In this study, the post-mitochondrial supernatant (PMS) was used as microsomal fraction. To prepare PMS, 1g of individual liver sample was homogenized in 5 ml of isolation buffer (250 mM Sucrose, 5mM Tris, 1 mM Mercapto ethanol and 0.5 mM Phenylmethylsulfonyl - fluoride, pH 7.4) and centrifuged as described by Adenuga (11) except that centrifugation at 100,000 Xg was omitted. The PMS was stored at -20°C until required for analysis.

This microsomal fraction was used for Glutathione (12), Catalase (13) and SOD (14) assay. Total Protein contents of liver samples were determined according to the procedure of Lowry *et al* (15) using bovine serum albumin as standard. Liver lipid peroxidation was assessed by measuring the thiobabituric acid-reactive (TBAR) products using the

procedure of Varshney and Kale (16). The data obtained were analyzed using student's t-test (Microsoft Excel inc. software).

RESULTS

The results of this investigation as presented in Table 1 indicate that protein undernutrition induced significant increase in lipid peroxidation ($P<0.001$) with significant reduction in catalase (CAT) activity ($P<0.001$), superoxide dismutase (SOD) activity ($P<0.05$) and glutathione (GSH) level ($P<0.05$) when compared with their well-nourished counterparts.

However, as presented in Table 2, liver regeneration due to partial hepatectomy provoked significant increase in lipid peroxidation ($P<0.05$) and CAT activity ($P<0.05$) but resulted in significant reduction in SOD activity ($P<0.05$) and GSH level ($P<0.05$) in PU rats when compared with their sham-operated counterparts.

DISCUSSION

PH is the most often used model to study liver regeneration, the molecular signaling and factors involved in cell proliferation. The determinants of hepatocyte proliferation during liver regeneration are highly complex, and so, the control of liver regeneration and the events involved in regulating the growth of the organ remain unknown (17)

There have been several reports on the levels of antioxidants in the liver of normal (well-fed) rats during regeneration. CAT, SOD and glutathione transferase and peroxidase activities are generally decreased while increased level of GSH in the hepatocytes isolated from regenerating liver have been reported (18).

Variable levels of LP in the various subcellular fractions have also been reported (19). For example, there is decreased lipid peroxidation in microsomes and increase in the plasma membrane and cytosolic fractions of normal (well-fed) rats following Partial hepatectomy (19). There are, however, little or no reports in the literature concerning changes in the levels

Table 1 : Lipid Peroxidation and other Biochemical Parameters of Well-fed and Protein Undernourished (PU) Rats

	Lipid Peroxidation (units/g tissue) $\times 10^5$	GSH (mg/g tissue)	Catalase Activity (units/g tissue) $\times 10^6$	SOD Activity (ng SOD/g tissue) $\times 10^3$
Well-fed Rats	2.513 $\pm$ 0.378	8.25 $\pm$ 0.710	1.825 $\pm$ 0.020	7.397 $\pm$ 0.179
PU rats	5.278 $\pm$ 0.220**	6.30 $\pm$ 0.440*	1.000 $\pm$ 0.084**	6.375 $\pm$ 0.263*

** $P<0.001$; * $P<0.05$; Well-fed rats were given diet containing 16% casein while PU rats were given diet containing 5% casein

Table 2 : Effect of partial hepatectomy (PH) on Lipid Peroxidative and Antioxidant Defense System of Protein-Undernourished (PU) Rats

	Lipid Peroxidation (units/g tissue) $\times 10^5$	GSH (mg/g tissue)	Catalase Activity (units/g tissue) $\times 10^6$	SOD Activity (ng SOD/g tissue) $\times 10^3$
Sham-operated PU rats	6.958 $\pm$ 0.027	4.98 $\pm$ 0.280	0.108 $\pm$ 0.014	4.391 $\pm$ 0.276
Partially Hepatectomised PU rats	7.850 $\pm$ 0.363*	3.39 $\pm$ 0.240*	0.325 $\pm$ 0.050*	1.882 $\pm$ 0.108*

* P<0.05; Both sham-operated PU and hepatectomised PU rats were fed with diet containing 5% casein. One sham-operated PU rat became moribund during the experiment.

of antioxidants in the liver of protein-undernourished rats during regeneration.

It is thought that LP might be a modulation of cell division, influencing both the initiation and cessation of mitosis in the regenerating liver (19). We report here, for the first time, that partially hepatectomised protein undernourished rats exhibit increased lipid peroxidation when compared with sham-operated protein-undernourished rats, suggesting increased ROS production following cell proliferation in the regenerating liver of PU rats. This observation in PU rats is consistent with the observation that PH induce an increase in LP in partially hepatectomised well-fed rats (19, 20).

Ordinarily, protein-undernutrition is accompanied with increased LP and reduced antioxidants levels (Table 1). These results on PH-induced increased LP taken together with the increased lipid peroxidation in PU rats suggest that liver regeneration induced by PH exacerbates LP in PU rats.

The antioxidant defense system of PU rats also responded to liver regeneration induced by partial hepatectomy with reduction in GSH level and SOD activity but increased (201%) CAT activity. This result suggests that CAT played a dominant role in the mopping up of ROS generated following liver regeneration in PU rats. Glutathione plays an important role in the protection of cellular components against oxidative damage and in the detoxification of many electrophilic compounds (21). The decreased levels of GSH may be due to increased rate of GSH oxidation under condition of increased prooxidant generation. The reduced activity of SOD may be a consequence of the reduced level of GSH which the former requires for its activity.

Our results on reduced GSH level and SOD activity in partially hepatectomised PU rats do not support the involvement of SOD in the mopping up of the generated ROS. The increased catalase activity, however, suggests the involvement of hydrogen peroxide in the increased lipid peroxidation in regenerating liver of PU rats. Further experiments on the

expression of antioxidant enzymes and direct measurement of the generated ROS are required to fully justify this postulate.

Conclusively, this investigation has provided evidence for an increased LP following PH in protein undernourished rats. It further revealed that PH exacerbates LP in PU rats and also suggests that catalase plays a major role in the mopping up of ROS generated following liver regeneration in partially hepatectomised PU rats.

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