Effects of severe malnutrition on oxidative stress in Wistar rats

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Oxidative stress was examined in three different types of induced-malnutrition such as marasmus, kwashiorkor and marasmic-kwashiorkor. Biomarkers such as superoxide dismutase (SOD), catalase and total protein were determined to assess oxidative stress. The activity of SOD and catalase showed that there was an increased oxidative stress in rats fed with the lowest protein diet (protein-calorie malnutrition group). The study confirmed that of the three states of malnutrition, oxidative stress was more pronounced in marasmic-kwashiorkor than in the other two groups.

Key words: Malnutrition, oxidative stress, antioxidants, enzymes, rats.

INTRODUCTION

Experimental protein deprivation in rats has been shown to reduce antioxidant cellular defense system in the liver and to enhance lipid peroxidation in plasma, red blood cell, heart, kidney and muscle (Touyz, 2000). Marasmus, kwashiorkor and marasmic-kwashiorkor are different types of malnutrition with different protein deprivation. Malnutrition can be described as inadequate intake of nutrients and its clinical symptoms can be classified into three main classes (that is Kwashiorkor, Marasmus and Marasmic-kwashiorkor) based on their nutritional deprivation (Touyz, 2000; Prata et al., 2007). The term “kwashiorkor” meaning the “disease the first child get when the second is on the way” in the Gia dialect of Ghana, was described by Williams for a previously recognized syndrome linked to malnutrition. In contrast to marasmus, the child afflicted with this syndrome characteristically has edema. Growth is stunted and wasting of skeletal muscle is apparent, but subcutaneous fat is maintained. Kwashiorkor in children frequently leads to death and terminal infections are invariably present (William, 1993; Scherbaum and Furst, 2000).

Marasmus is a chronic condition resulting from a deficiency in total energy intake, whether the source of energy is protein, fat or carbohydrate. The marasmic individual has consumed all the reserves of protein and energy and is thus severely wasted in skeletal muscles as well as fat depots. A child with this syndrome is generally inactive and underweight, with prominent ribs and head that appears disproportional in size. Vital muscle functions are impaired, as is immune function and subcutaneous fat is absent (Scherbaum and Furst, 2000).

Marasmic-kwashiorkor is a condition characterized by features of both marasmus and kwashiorkor and is a form of severe protein-calorie malnutrition (PCM) that occurs when stress is superimposed on a chronically ill, starved patient. Body fat stores as well as both somatic and visceral protein stores are depleted. This condition is marked by a high incidence of life threatening complications. Immunocompetence is lowered. The prognosis is very poor, because of the high risk of infections and the poor wound healing (Scherbaum and Furst, 2000; Touyz, 2000).

Protein-calorie malnutrition (PCM), also referred to as protein-energy malnutrition, has long been recognized as a common problem, especially in children in the developing countries, whose inadequate nutritional intake is deficient for socio-economic reasons (Manary et al., 2000). The term PCM covers a whole range of deficiency states, from mild to severe and has been defined as “a range of pathological conditions arising from coincident lack, in varying proportions, of protein and calories,
occurring most frequently in infants and young children and commonly associated with infections (WHO and FAO, 1973; Manary et al., 2000). This definition emphasizes the important concepts that inadequate intake of both protein and energy-yielding food can lead to PCM and that various forms of malnutrition are inter-related. The individual initially responds to deprivation of protein or calories or both, by adaptation and only when the deprivation continues does an eventual physiological breakdown occur, marked by symptoms and clinical signs. Mild or moderate PCM diminishes the rate of increase in size and growth in young children, whereas the severe PCM resulting from prolonged nutritional deprivation comprises a range of conditions that can be put into three main classes, Kwashiorkor and marasmus, which form the two extremes of the spectrum and are caused by protein and energy deficiency respectively and the intermediate class, marasmic-kwashiorkor. Manary et al. (2000) suggested that the symptoms of marasmus and kwashiorkor are two facets of the same disease entity, the final outcome being determined by the individual’s capacity to adapt to nutritional stress. It has also been proposed that an adequate adrenocortical response is needed for satisfactory adaptation to the nutritional deprivation leading to marasmus, whereas failure to adapt gives rise to kwashiorkor.

Oxidative stress is a general term used to describe the steady state level of oxidative damage in a cell, tissue or organs caused by an imbalance between the production of reactive oxygen species and biological systems ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbance in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA (Schafer and Buettner, 2001). It has been reported that the effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidation stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis (Sohal, 2002).

A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted by oxido-reduction reactions with transition metals or other redox cycling compounds including quinines into more aggressive radical species that can cause extensive cellular damage (Valko et al., 2005). Most of these oxygen-derived species are produced at a low level by normal aerobic metabolism and the damage they cause to cells is constantly repaired. However, under the severe levels of oxidative stress that cause necrosis, the damage causes ATP depletion, preventing controlled apoptotic death and causing the cell to simply fall apart. (Lee and Schacter, 1999). In humans, oxidative stress is involved in many diseases, such as atherosclerosis, parkinson’s disease and alzheimer’s disease, but it may also be important in prevention of ageing by induction of a process named mitohormonesis. Reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens. Reactive oxygen species are also used in cell signaling (Touyz, 2000).

Oxidative stress is induced by the overproduction of reactive oxygen species (ROS), which oxidize lipids, proteins and DNA and lead to cell membrane destruction, resulting in several diseases. It has been suggested that such disease as heart disease, stroke, diabetes, vascular disease, cancer and even early aging are induced by oxidative stress (Sohal et al., 2002; Ruano-Ravina et al., 2006). It has also been suggested that a high oxidative stress is usually accompanied with decreasing antioxidant capacity and this tendency is obvious in cancer patients. It has also been stated that vascular endothelial cells promptly respond to oxidative stress by synthesizing several oxidative stress-inducible proteins and stimulating antioxidant enzymes apparently to protect themselves from the toxic effects of stress (Touyz, 2000; Ruano-Ravina et al., 2006).

It seems clear that oxidative stress can lead to the formation of the noxious free radicals \( \cdot O_2 \) and \( \cdot OH \) as well as \( H_2O_2 \), which, can under certain circumstances lead to the formation of \( OH \). In turn, these reactive oxygen species negatively affect tissues in a variety of ways, including DNA damage, impairment of mitochondrial respiration, and direct parenchyma impairment (Halliwell, 2000). Oxidative processes can affect DNA indirectly through protein oxidation or lipid oxidation or directly by oxidation of DNA (Halliwell, 2000). Indirect mechanisms leading to DNA damage include protein oxidation, which could alter repair enzymes and DNA polymerases. When reactive oxygen species interact with lipids, the resulting lipid peroxidation products might then subsequently react with DNA, inducing mutations (Nozik et al., 2005). Similarly, reactive nitrogen species can also damage proteins needed for oxidant defense or DNA repair or induce lipid peroxidation resulting in further cell damage to lipids, protein or DNA. The most important mechanisms of DNA damage, however, are believed to involve direct attack of oxidants on individual nucleotides in DNA. Guanine is the DNA base most susceptible to oxidative attack (Halliwell, 2000).

Antioxidants either in form of vitamins or enzymes are necessary in combating the adverse effects of reactive oxygen species. Antioxidants are classified into two broad divisions depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in cell...
cytoplasm and the blood plasma, while lipid-soluble antioxidants prevent cell membranes from lipid peroxidation (Sies, 1997). These compounds may be synthesized in the body or obtained from the diet (Vertuani et al., 2004). The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed throughout the body. The relative importance and interactions between these different antioxidants is a complex area with the various metabolites and enzymes having synergistic and interdependent effects on one another (Halliwell, 2000; Vertuani et al., 2004). The action of one antioxidant may depend on the proper function of other members of the antioxidant system. The amount of protection provided by any one antioxidant therefore depends on its concentration, its reactivity towards the particular reactive oxygen species being considered and the status of the antioxidants with which it interacts (Vertuani et al., 2004). The study therefore is an attempt to elucidate the role of oxidative stress in different types of induced malnutrition using animal model.

MATERIALS AND METHODS

Animal care

Male Wistar rats weighing 35 - 40 g were selected randomly into 4 different groups (one control group and three experimental groups A, B and C). Each group comprised of 6 rats and was housed separately in a wire mesh cage, under standard conditions (temperature 25 - 29°C; 12 h of light and 12 h of darkness cycle). The control group that is, the group that consumed the complete diet ad libitum. Group C, kwarsiokork energy-deficient group that consumed the complete diet in restricted daily quantities. All the animals were given access to water and they were fed for six weeks.

The study received the approval of the Ethical Committee of Ladoke Akintola University of Technology, Ogbomoso, Nigeria and all animals used in the study were treated in accordance with the principles of laboratory animal care as indicated in the Guide for the Care and Use of Laboratory Animals of the National Institute of Health (1978).

Blood collection

After six weeks of feeding, the rats were sacrificed using cervical dislocation and blood was collected directly from the heart of the animals through 5 ml syringe into EDTA bottles for white blood cell count and packed cell volume and into plain bottles in order to obtain serum for biochemical analysis.

Serum preparation

The blood collected into the plain bottles was allowed to clot, then centrifuged in 4000 x g and the supernatant was collected into another plain bottle for analysis.

Determination of catalase activity

Catalase activity was determined as described by Nozik et al. (2005).

Determination of superoxide dismutase (SOD) activity

SOD activity was determined by the method of Misral and Fridovich (1972).

Determination of total protein

Total protein is determined according to the standard method of lowry (1951).

Statistical analysis

The results are expressed as mean ± SEM and P value of P < 0.05 was considered to be statistically significant.

RESULTS

Figure 1 shows the activity of SOD in control and experimental groups. The SOD activity was significantly lower in the rats with marasmic-kwashiokor when compared with the control group. However, there was no significance difference (P > 0.05) in SOD activity in the marasmic, kwashiokor and marasmic-kwashiokor rats.

Table 1 shows catalase activity (IU/min) in marasmic, kwashiokor and marasmic-kwashiokor Wistar rats. It shows that catalase activity was significantly (P < 0.05) higher in the marasmic-kwashiokor rats than the other two experimental groups and control group. There was
Table 1. Mean ± SEM of catalase activity in marasmic-kwashiokor, kwashiorkor and marasmic Wistar rats.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Marasmic-Kwashiokor group</th>
<th>Kwashiorkor group</th>
<th>Marasmus group</th>
</tr>
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<tbody>
<tr>
<td>Catalase activity</td>
<td>0.49 ± 0.19</td>
<td>0.65 ± 0.27*</td>
<td>0.21 ± 0.05</td>
<td>0.12 ± 0.07</td>
</tr>
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*significant

Figure 2 shows that there was a significance difference (P < 0.05) in the level of total protein between marasmic, kwashiorkor and marasmic-kwashiokor rats and control group being higher in control and lower in experimental groups.

Table 2 shows the hematological parameter in marasmic, kwashiorkor and marasmic-kwashiokor in Wistar rats. It shows that there was a significance (P < 0.05) difference in the hematological parameters of marasmic, kwashiorkor and marasmic-kwashiokor rats. There was a decreased WBC count in these groups of rats compared to the control group.

DISCUSSION

Nutritional stress in the form of deficient protein condition is quite prevalent in developing countries (Crogan and Pasvogel, 2003). Protein malnutrition disorders include growth failure, hypoproteinemia, edema, fatty liver and decreased immune defense in humans and animals (Keusch, 2003). Protein-deficient diet intake has been shown to influence the activity of drug metabolizing enzymes as well as antioxidant enzymes (Nozik et al., 2005). Feeding of a protein-deficient diet to rats has been shown to increase lipid peroxidation and induce significant changes in activities of catalase, glutathione peroxidase and superoxide dismutase. In this current study, protein-deficient rats showed significant increase in catalase activity and a significant inhibition in the levels of SOD activity suggesting that the rats were potentially susceptible to oxidative stress. Catalase activity was enhanced in marasmic-kwashiokor rats than in the other two groups. This is in concomitance with the earlier work of Sidhu et al. (2004) indicating that malnutrition possibly caused alterations in antioxidants enzymes activities. Therefore, it may be appropriate to say that, the higher catalase activity in malnourished rats may reflect the
chronic exposure of the cells to oxidativie stress.

The superoxide dismutase (SOD) activity was significantly lower in marasmic-kwashiokor than in marasmic and kwashiokor. This may be due to the fact that marasmic-kwashiokor (PCM) is a more severe form of malnutrition. It has been proposed that free radical-mediated tissue damage may be involved in malnutrition mainly because of the inadequate protective and repair mechanisms in protein-deficient animals or humans. The essential role of ROS with respect to the aetiology of kwashiokor was first published in 1987 and has been repeatedly advocated since then (Scherbaum and Furst, 2000). As a measure of the intracellular depletion of antioxidant capacity, low antioxidant status was reported in kwashiokor. Investigations of enzymes responsible for maintaining glutathione in the reduced state and studies in response to oxidative stress found increased activity of G6PD and showed that impaired antioxidant status and decreased proportions of red cell phospholipids were found in different types of malnutrition.

Protein-deficient treatment groups, showed a highly significant reduction in total protein level compared to the control group which is in agreement with earlier reports (Davenport et al., 1994; Prata et al., 2007). The level of serum total protein of the kwashiokor-marasmus group was significantly lower than the kwashiokor, marasmus and control groups. These findings suggest an altered protein and antioxidant status in protein-energy malnutrition. In this study, experimental malnutrition is associated with concomitant sign of intestinal free radical damage and altered protein transport. This is in agreement with the works of earlier authors (Darmon et al., 1993) suggesting that oxidative stress is partly responsible for the intestinal dysfunction observed in malnutrition. Anemia has been well documented in kwashiokor, marasmus and protein-calorie malnutrition (Scherbaum and Furst, 2000) and it is suggested that this may be secondary to depressed erythropoesis and decreased red cell survival. The present study shows that there was a reduced level of haematocrit in the malnourished group compared to the control.

**Conclusion**

This study showed that protein-deficient rats demonstrate increased oxidative stress and that the degree of oxidative stress depends on the severity of protein deficiency. Further studies that will examine antioxidant enzymes, vitamins and electrolytes in details are recommended.

**REFERENCES**


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