ORIGINAL ARTICLES

Proteins and Liver Function Changes in Rats Following Cumulative Total Body Irradiations

CR Nwokocha[1], M Nwokocha[1], P Mounmbegna[2], J Orhue[3], O Onyezuligbo[2], EH Olu-Osifo[2], E Okojie[2], E Asuquo[2], T Ejimofor[2], N Ikenna[2], M Judith[2]

ABSTRACT

Objective: *Total body irradiation (TBI) is a choice therapy for the management of some malignancies; it is also a major cause of oxidative stress. The aim of this research is to sequentially document the effect of total body radiation on body function utilizing the sequential changes in liver function enzymes and proteins in rats.*

Methods: *Serum protein and liver enzymes were assessed using kits in rats exposed to total body radiations of 1.27 Gy/minute in cumulative doses to the fourth radiation at five-day intervals.*

Results: *Aspartate aminotransferase (AST), alanine transaminase (ALT) and serum protein were significantly (*$p < 0.05$*) elevated with increasing radiation. No significant differences between experimental and control groups for bilirubin concentrations were noted at any time. Serum levels of albumin were significantly (*$p < 0.05$*) increased with the first to third radiation exposures but reduced at the fourth cumulative dose exposure.*

Conclusion: *Variations are associated with acute stress, inflammation which could be due to non-specific stress reaction, while fluctuations could arise as a result of tolerance and repair within the liver. These tests are significant for diagnosis of radiation-induced injury and can be important for evaluation of its severity and correct management.*

Keywords: Albumin, ALT, AST, bilirubin, liver enzymes, protein, radiation injury, total body irradiation

Proteínas y Cambios de Función Hepática en Ratas Tras Irradiación Corporal Total Cumulativa

CR Nwokocha[1], M Nwokocha[1], P Mounmbegna[2], J Orhue[3], O Onyezuligbo[2], EH Olu-Osifo[2], E Okojie[2], E Asuquo[2], T Ejimofor[2], N Ikenna[2], M Judith[2]

RESUMEN

Objetivo: *La irradiación corporal total (ICT) es una terapia de elección para el tratamiento de algunos tumores malignos. También es una de las causas principales del estrés oxidativo. El objetivo de esta investigación es documentar el efecto de la radiación corporal total sobre las funciones corporales utilizando los cambios secuenciales en las enzimas de la función hepática y las proteínas en las ratas.*

Métodos: *Se evaluaron las proteínas séricas así como las enzimas hepáticas mediante el uso de kits en ratas expuestas a radiaciones corporales totales de 1.27 Gy/minuto en dosis cumulativas hasta la cuarta radiación con cinco días de intervalo.*

Resultados: *La aspartato transaminasa (AST), la alanina aminotransferasa (ALT) y la proteína sérica fueron elevadas significativamente (*$p < 0.05$*) con el aumento creciente de la radiación. No hubo*

From: [1]Department of Basic Medical Sciences, The University of the West Indies, Kingston 7, Jamaica, [2]Department of Physiology and Biochemistry, Madonna University, Okija, Nigeria and [3]Department of Biochemistry, University of Benin, Nigeria.

Correspondence: Dr CR Nwokocha, Department of Basic Medical Sciences, The University of the West Indies, Kingston 7, Jamaica. E-mail: chukwuemekanwokocha@uwimona.edu.jm

diferencias significativas entre el grupo experimental y el grupo control, observándose concentraciones de bilirrubina todo el tiempo. Los niveles séricos de albúmina aumentaron significativamente ($p < 0.05$*) con la primera de tres exposiciones a la radiación, pero experimentaron una reducción a partir de la cuarta exposición de dosis cumulativa.*

Conclusión: *Las variaciones están asociadas con estrés agudo e inflamación que podría deberse a una reacción a estrés no específico, mientras que las fluctuaciones podrían surgir como resultado de la tolerancia y la regeneración dentro del hígado. Estas pruebas son importantes para el diagnóstico de lesiones inducidas por radiación, así como para la evaluación de la severidad y el tratamiento correcto de las mismas.*

Palabras claves: Albúmina, ALT, AST, bilirrubina, enzimas hepáticas, proteína, lesión por radiación, irradiación corporal total

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INTRODUCTION

Whole body exposures to any form of radiation are known to alter the general physiology of the animal (1). Ionizing radiation inflicts its adverse effects through the generation of oxidative stress that unleash large-scale destruction or damage of various biomolecules (2, 3). These free radicals react with body tissues and generate lipid peroxidation, DNA lesions and enzyme inactivation, all of which are mediators of radiation damage.

Total body irradiation (TBI) has been used in the clinical treatment of many malignancies (4−7) to produce sufficient immunosuppression and prevent allograft rejections (5). It is a choice of management for hepatocellular carcinoma, a leading cause of cancer-related deaths (8) and other tumours (9, 10) but ionizing radiations are able to alter micro-environments producing untargeted effects on normal tissues; these effects can enhance tissue metastasis and cancer (11).

As such, an association has been established between ionizing radiation and malignant disease (12−14). Among such are various liver diseases, presenting with tender hepatomegaly, hyperbilirubinaemia and ascites (15, 16); it is thought that these could be prevented if the total body irradiations are reduced or even avoided.

Long term radiation-associated changes, especially in various occupation-exposed groups (17, 18), are presumably a result of some change in liver metabolism. Irradiation of the liver induces upregulation of the genes of the main proinflammatory chemokines, probably through the action of locally synthesized proinflammatory cytokines (19−21). Oxidative stress is a condition associated with an increased risk of cellular damage induced by oxygen and oxygen derived oxidants commonly known as reactive oxygen species [ROS] (22−24). The increase in oxidative stress in the liver is highlighted by increases in concentrations of thiobarbituric acid-reactive substances (TBARS), a marker of lipid peroxidation and decreases in superoxide dismutase, glutathione peroxidase (GPx) activity, activation of the stress-inducible haemoxygenase-1 (HO-1) gene (25, 26) and reduced glutathione concentration [GSH] (22, 27). But others have reported no changes in levels of TBARS following exposure to radiation; they opined that exposure played no vital role in the serum profiles alteration (28−30). Yamazaki *et al* (31) had reported that with TBI, there is a transient elevation of hepatocyte growth factor (HGF) which acts as a potent organotropic factor in the regeneration of various organs following injury. The capacity to proliferate in response to injuries is a major characteristic of the liver (32).

Against this background of the clinical usefulness of total body irradiation and the attendant effects on the body organs, with special emphasis on the liver, this study aims to sequentially document the effect of cumulative total body radiation on body function utilizing the sequential changes in the liver enzyme functions in rats.

SUBJECTS AND METHODS

Male Wistar rats weighing between 180 and 250 g aged 5−7 weeks were obtained and kept at the Animal House of the Faculty of Basic Medical Sciences for this study. During the entire treatment period animals were kept at room temperature of 28 ± 2 °C with 12 hours light/dark cycles. The rats were fed with standard rat chow and water *ad libitum*.

The animals were randomly assigned into five cages of five each, with group 1 serving as control and receiving no radiation, group 2 received radiation once, twice for group 3, thrice for group 4 and four times for group 5. The time interval between total body radiations was five days. Thus, in the test group, a set of five rats received radiation once, twice, thrice and four times, which may be regarded as test groups one, two, three and four, respectively.

An X-ray generator (200 kV and 20 mA, Villa SystemiMedicali, Italy) with a filter (0.5 mm Cu and 0.5 mm Al) was used for the experiments. The dose of radiation was determined using a dose metre at a dose rate of 1.27 Gy/minute. The experiments were conducted according to international protocols for the use of animals in experimental studies and the study was approved by the Faculty of Medical Sciences Ethics Committee, Madonna University, Nigeria..

Biochemical analysis of serum

Plasma biochemical analysis was performed within 24 hours of blood collection. Blood was collected from the animals sacrificed under pentobarbital (50 mg/kg bodyweight) anaesthesia post TBI. The blood samples were centrifuged after allowing for clotting (1−3 hours) at 2000 revolution per minute for 15 minutes. The sera were assayed for serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using randox kit following standard methods of Reitman and Frankel (33) [colorimetric], while albumin, total protein and bilirubin were determined in accordance with the principles described by Tietz (34).

Statistical analysis

The data are presented as the mean ± standard error of mean (SEM) for the individual groups. The results were analysed using GraphPad Prism software version 5 (GraphPad Software, San Diego, California, USA). Student's paired *t*-test and analysis of variance (ANOVA) were used to compare means where *p*-values were significant. A *p*-value of 0.05 was considered significant.

RESULTS

Results for the liver function enzyme values are listed in the Table; there were also no significant differences between the study groups for bilirubin. The albumin values were significantly ($p < 0.05$) increased with the first, second and third radiation; values for the fourth radiation, though decreased, were not statistically significant. There were also no significant differences in protein levels between the control and animals in the first, second and third radiations, however, with the fourth radiation, protein levels were significantly ($p < 0.05$) elevated.

The values for AST were significantly ($p < 0.05$) elevated for the first and third radiation exposures, there was a significant ($p < 0.05$) decrease with values obtained with the second radiation exposures, while the fourth exposure did not show any significant difference.

The values for ALT showed a gradual increase among the radiation exposed groups when compared with the controls. The values for the second to the fourth radiation exposures were statistically significantly ($p < 0.05$) elevated.

DISCUSSION

In the present study, we used the rodent model to test the time-course effects of continuous or consecutive exposure to X-ray radiation on liver function tests using enzyme markers for hepatocyte. The whole body exposure approach could stimulate any tissue; however, there were significant differences between experimental and control groups for some of the measured indices.

Total serum proteins are, diagnostically, of relative importance in assessing the state of health of an organism, their increase appearing especially in inflammatory processes and tissue dysfunction (35). Holten and Christiansen (36) had reported that irradiation causes decreased serum protein and albumin levels. Our observations were that serum protein levels varied within normal ranges but were significantly elevated with the fourth cumulative radiation exposure; albumin on the other hand was reduced only after the fourth radiation exposure. Moulder *et al* (37) have reported that radiation leads to proteinuria, which is associated with low serum albumin and total protein (38). We had earlier reported a transient elevation in cholesterol and lipid levels following cumulative doses of radiation (39, 40); here our observations of elevated proteins can only point to increased inflammatory processes and tissue dysfunction showing the role or involvement of the liver in this radiation injury.

The reduced GSH concentrations can increase stress induced oxidation since its direct radical scavenging properties will be reduced (41), but an increase in the HO-1 can counteract these pro-oxidant conditions. Haemoxygenase-1 has a protective role in inflammation, its production is induced by haem, which may be derived from destabilized membrane bound tissues or even from damaged erythrocytes (42), it then acts by removal of free haem (a pro-oxidant) and the production of bilirubin, which is considered as a potent antioxidant (42−44). Haemoxygenase-1 also catalyses oxidative breakdown of haem molecule to carbon monoxide [CO; potent anti-inflammatory properties] (45, 46), ferrous oxide (Fe^{2+}) and biliverdin, which is subsequently reduced to bilirubin by biliverdin reductase (47, 48).

Our observations were that bilirubin values fluctuated within normal physiological limits; the elevations were not statistically significant. Our results do not suggest hepatic

Table: Liver function tests values in rats following total body radiation, presented as mean ± SEM

(mmol/l)	control	1st radiation	2nd radiation	3rd radiation	4th radiation
bilirubin	8.80 ± 3.20	11.20 ± 4.76	8.80 ± 3.61	12.4 ± 5.50	11.8 ± 3.76
albumin	3.09 ± 0.31	3.63 ± 0.20*	3.59 ± 0.51*	4.53 ± 0.25*	2.87 ± 0.56
total protein	3.44 ± 0.23	3.54 ± 0.20	3.16 ± 0.30	3.55 ± 0.29	3.80 ± 0.25*
ALT (IU)	10.76 ± 0.71	11.22 ± 0.07	13.06 ± 1.19*	14.06 ± 0.10*	14.37 ± 0.12*
AST (IU)	4.36 ± 0.25	4.97 ± 0.22*	3.77 ± 0.25*	4.80 ± 0.28*	4.58 ± 0.53

* = $p < 0.05$ compared with control values.
ALT − alanine aminotransferase; AST − aspartate aminotransferase

damage following irradiation as may have been suggested by some researchers (49−52). We do not know if the experimental designs and type of animals used may have contributed to this, but our results are in agreement with those of Baker *et al* (53), that the low serum albumin and total protein is as a result of proteinuria. Our observation of some form of rebound from the measured indices may be a form of homeostatic mechanisms within the body as it seems to be better equipped to chronic rather than acute stressors like radiation; an adjustment of these body systems and mechanisms can be a method of adaptation to stressors.

The present study showed that liver function was compromised over the course of study following TBI. The radiation-induced changes in liver function using AST levels as enzyme markers for hepatocyte health are modest. But with ALT, we observed a continuous elevation with cumulative doses of radiation. The liver function tests are very important for diagnosis, evaluation of severity and correct management of radiation-induced injury even with the fluctuations observed. We were not able to measure proteinuria, ferritin levels as well as prothrombin time and international normalized ratio (PT/INR) levels, also oxidative stress parameters (TBARS, GPx HO-1 *etc*) which are useful adjuncts to determining the effects on the liver function tests in the course of this work. We were also not able to determine the quantity of cumulative radiation dose received by the animals. But the fluctuations in liver function observed could arise due to some tolerance of the liver to ionizing radiation. The tolerance seen in the liver could be due to the high damage induced repairs especially peculiar to cells in the liver (54−56) to non-specific stress reaction.

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