Effects of total body irradiation on fatty acid and total lipid content of rats

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Abstract: We examined time-dependent changes in plasma lipids of rats given total body irradiation (TBI) with X-rays at 3 Gy. for consecutive periods. Animals were exposed to x ray radiations consecutively for 20 days at 5 day interval thereafter five animals were picked at random and sacrificed (5, 10, 15 and 20 days after beginning the exposure). The triacylglycerols and total cholesterol serum levels were significant differences between control and experimental groups after the first exposure (5 days), values for the triacylglcerols were significantly higher with the second (day 10) and third (day 15) radiation exposures but not with the fourth radiation exposures (day 20) ($P<0.05$). However, the serum cholesterol values were not found to be significant with the second and third exposures but with the fourth exposure (day 20) ($P<0.05$). The serum HDL-C concentrations were not significantly different between control and experimental groups at any time analyzed. But the LDL cholesterol was found to decrease on days 5 and 20 of the experimental period. Our results indicate that the applied long term exposure to x rays ionization radiations exposure may induce slight but statistically significant alterations in some serum lipids profile of rats, within the physiological range. The mechanisms for the effects of these ionizing radiations on serum lipid profile are not well understand yet, we suggest that the changes could be due to some non-specific stress reactions. The consequences of our observation are not known yet, but could point to some possible clinical intervention.

Keywords: Total body irradiation, lipids, Cholesterol, Triacylglycerol.

INTRODUCTION

Researchers have reported some seemingly contradictory or inconsistent findings of radiation health effects risks for exposed populations (Hardell & Sage, 2008) which may be due to applied methods, differences in sensitivities or even epidemiological analysis of those populations, (Nussbaum & Köhnlein, 1994). One persistent notion in many reviews of low-dose effects is the hypothesis of reduced biological effectiveness of fractionated low-dose exposures, compared to that of the same acute dose (Nussbaum & Köhnlein, 1994). Evidence abounds that environmental and artificial magnetic fields which will include X-rays and ionizing radiations have significant impact on cardiovascular systems of animals and humans (Szmigielski *et al.*, 1998, Sastre *et al.*, 2000, Graham *et al.* 2000, McGale & Darby 2005). Adams *et al.* (2003) reported some damage to the coronary arteries in patients receiving RT and in experimental animals leads to increased risk of cardiovascular diseases, possibly as a result of confounding factors (Little *et al* 2008). Wong *et al.* (1986) also reported changes in cholesterol concentrations associated with long term radiation in Japanese atomic bomb survivors, also in various occupationally-exposed groups (Howe *et al.*, 2004, Azizova & Muirhead, 2009), this they assumed was due to liver metabolism changes. McGeoghegan *et al.* (2008) associated exposure to ionizing radiation with mortality from circulatory system disease which is not consistent with any simple causal interpretation. Yukiko *et al*, 2010, reported that stroke and heart diseases are associated with elevated exposures (above 0.5 Gy) leading to death as seen in many survivors of atomic bombs and cancer.

Zielinski *et al* 2009 reported a strong positive association between radiation doses at means whole body radiation dose was 8.6 mSv for men and 1.2 mSv for women and the risk of CVD mortality. Hoel (2006) also reported a measurable increases in cardiovascular disease mortality and arterial endothelial damage from both neutron and, to a lesser extent, gamma exposures at doses greater than 0.5 Sv. Metz-Flamant *et al.* (2009) had argued that While cardiovascular risks associated with high level of ionizing radiation are well-established, long-term effects of low and medium levels of exposure, between 0 and 5 gray (Gy), on the cardiovascular system are debated. On the other hand, beneficial effects of extremely low frequency electromagnetic fields (ELF-EMF) have also been reported (Torres-Duran *et al.*, 2007). In diet-induced hypercholesterolemic rabbits, pulsed of EMF lowers total cholesterol and triacylglycerols levels (Luo *et al.*, 2004); similar results have been found in rats (Bellossi *et al.* 1998) and mice (Kumosani & Qari, 2003) both fed on control diets. The likely mechanisms for such effects of low dose and/or chronic radiation exposures on cardiovascular disease are not clear but Little *et al.* (2008) has suggested endothelial cell damage leading to some inflammatory response, inflammatory diseases like

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atherosclerosis can lead to myocardial infarction. Little *et al*. (2010) in a recent paper proposed a monocyte cell killing in the intima and some form of somatic mutation giving rise to more inflammation.

Metz-Flamant *et al.* (2009) had argued that given the multifactorial origin of cardiovascular diseases and the lack of a clear pathophysiologic mechanism, epidemiological results have to be carefully interpreted. Further research should be conducted in this area to deduce more biological evidence that will give a better understanding of course of events leading to theses damages, as such it is important to understand the time-course effects of X ray radiation on serum lipid levels in biological models, exact knowledge of pathophysiological mechanisms if any of radiation induced cardiovascular damage after radiotherapy which might be useful for the detection of abnormal values and their early management especially in the asymptomatic patients. (Hudecová, *et al.* 2008) this is because radiation therapy continues to pose a risk for delayed-onset cardiovascular disease (Anderson & Sawyer, 2008). The aim of the present study was to investigate using Wister rats, the time-course effects low dose and/or chronic radiation exposures of X-ray on serum lipid concentrations.

MATERIALS AND METHODS

25 Wistar albino rats with weights ranging from 180 to 200g were used for the experiment. The rats were allowed to acclimatize for a period of two weeks in a room with a constant temperature of 23 ± 1°C and a 12 h light-dark cycle during which they had free access to rodent chow and drinking water throughout the experimental period. Rats were subjected to TBI via x-rays at a dose of 3 Gy in a chamber without anesthesia. X-ray irradiation was performed using a soft x-ray unit.

On commencement of the experiment, five rats picked at random were separated from the total number of rats (25) and this group was used as the control subjects. The control subjects were not exposed to radiation. While the remaining, 20 rats, the test subjects, were irradiated.

On the first day, the test group received equal amount of total body irradiation (TBI). Blood samples were collected from 5 rats selected at random after radiation exposures, the 15 rats remaining from the test group were irradiated with the same amount of radiation as on the first day, and blood samples collected from 5 rats picked at random. After another five days, now 10 rats remaining were irradiated as usual and blood samples collected from any five. The last 5 rats were irradiated 5 days after the previous and their blood samples were collected.

The rats were sacrificed under pentobarbital anesthetize post-TBI. Their blood was taken from the abdominal aorta, immediately centrifuged at 5000 × *g* for 3 min, and the plasma was prepared.

Thus, in the test group, a set of 5 rats received radiation once, twice, trice and four times, which may be regarded as test groups one, two, three and four respectively. All procedures were carried out in accordance with the guidelines for the care and use of laboratory animals in our institute.

Biochemical assay

Triglycerides, HDL-cholesterol and Total Cholesterol were analyzed enzymatically using kits obtained from Randox Laboratories Limited, Crumlin, UK. Plasma LDL-cholesterol was determined from the values of total cholesterol and HDL- cholesterol using the friedwald's formula (1972), LDL-C = TC–(HDL-C)–(triglyceride/5).

STATISTICAL ANALYSIS

The data are presented as the mean ± standard error of mean (SEM) for the individual groups. Normally distributed data were analyzed using parametric tests, i.e. Student's paired t-test and analysis of variance (ANOVA), a value of $p<0.05$ was considered significant. All statistical analyses were performed using the computer program Microsoft excel and Origin 5.0.

RESULTS

Serum lipid concentrations

There were statistical significant differences ($P<0.05$) between control and experimental groups for triacylglycerols (113.44 ± 12.37 vs 53.20 ± 7.62) and total cholesterol serum levels, (132.80 ± 11.21 vs 95.83 ± 7.19) after the first exposure (day 5), values for the triacylglcerols were significantly higher with the second (day 10) (46.13±8.35) and third (day 15) (82.54 ± 4.97) radiation exposures but not with the fourth (day 20) radiation exposures ($P<0.05$) (fig. 1). However, the cholesterol values were not significant with the second and third exposures but with the fourth exposure (101.82 ± 6.02) ($P<0.05$) (fig. 2). There were no significant differences between experimental and control groups for HDL-C concentrations serum levels at any time analyzed (fig. 2).

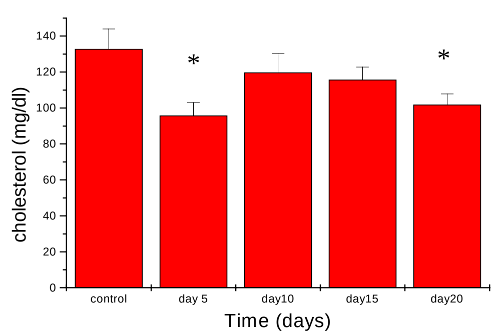

Fig. 1: Cholesterol serum levels in control group and day 5, day10, day15 and day20 after successive exposure to X-ray. *Significantly different from control group (p <.05).

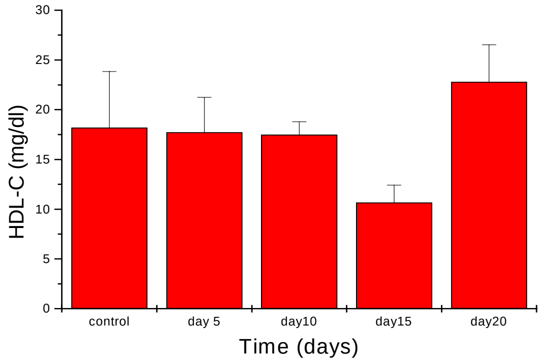

Fig. 2: HDL-cholesterol serum levels in control group and day 5, day 10, day 15 and day 20 after successive exposure to X-ray. *Significantly different from control group (p<.05).

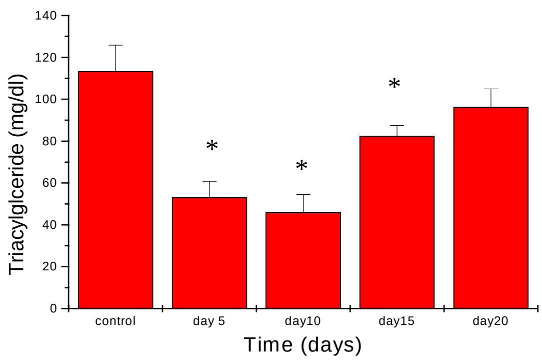

Fig. 3: Triacylglyceride serum levels in control group and day 5, day 10, day 15 and day 20 after successive exposure to X-ray. *Significantly different from control group (p<.05).

For the LDL cholesterol levels, values observed were lower for day 5 and day 20; these values were computed from the Friedewald 1972 formular (fig. 4).

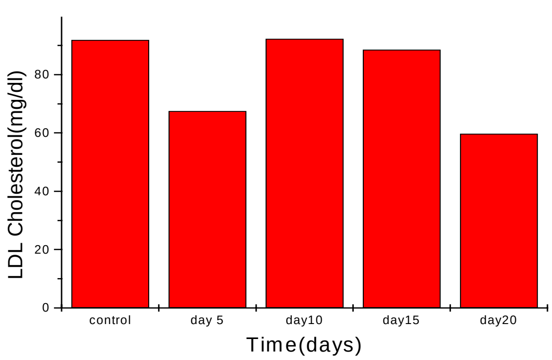

Fig. 4: LDL cholesterol serum levels in control group and day 5, day10, day 15 and day 20 after successive exposure to X-ray. *Significantly different from control group (p<.05).

DISCUSSION

In the present study, we used the Wister rats to test the time-course effects of continuous or consecutive exposure to x ray radiation on serum lipids. The whole body exposure approach could stimulate any tissue; however, There were significant differences between control and experimental groups for total cholesterol and triacylglycerols serum levels, after the first exposure (5 days), values for the triacylglcerols were significantly higher with the second and third radiation exposures but not with the fourth radiation exposures (P<0.05). However, the serum cholesterol values were not found to be statistically significant with the second and third exposures but with the fourth exposure (P<0.05). In comparison, Torres-Duran *et al.* (2007) had observed no changes in total cholesterol and triacylglycerol serum levels, but higher values for HDL-C and free fatty acids at 48 and 24 hours when the exposed groups were compared with the control groups in her study, our results are partially in accordance with. Our results is in agreement with Bellossi *et al. (*1998, 1996) who had observed that Twenty-four hours after the end of the exposure to the PMF cholesterol and triglyceride plasma levels decreased but a latter increase after 48hours in the triglyceride level the rats' livers were heavier. Normal values were observed 48 and 96 hours after the end of exposure respectively for cholesterol and triglycerides, the concluded that these alterations may be due to a reversible accumulation of either triglycerides or of their precursors. Furthermore, Luo *et al.* (2004) has reported significantly decreased cholesterol and triacylglycerols but elevated HDL-C values when hyperlipidemic diet-induced female rabbits were exposed to a 15 Hz, 0.4 mT, 10 hours/day by 8 weeks.

Harakawa and colleagues (Harakawa *et al.,* 2005) reported a significantly decreased free fatty acids and triacyglycerol plasma levels with exposure, while Kula *et al.* (1999) reported a reduction in serum total lipids in human with increased exposures.

There were no significant differences between control and experimental groups for HDL-C concentrations serum levels, at any time analyzed, our results indicate that the applied ionization radiations exposure may induce slight but statistically significant alterations in some serum lipids profile of rats, within the physiological range. The mechanisms for the effects of these ionizing radiations on serum lipid profile are not well understand yet, we suggest that the changes could be due to some non-specific stress reaction. The consequences of our observation are not known yet, but could point to some possible clinical intervention, further studies may rationalize this.

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