EFFECT OF IMMOBILISATION STRESS ON LIPID PEROXIDATION AND LIPID PROFILE IN RABBITS

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ABSTRACT

Stress is one of the basic factors in the aetiology of a number of diseases such as atherosclerosis, coronary heart disease, aging, liver disease etc. Hence this work was designed to study the effect of stress in the form of forced immobilisation stress on lipid peroxidation and lipid profile in rabbits. The study was conducted in 25 healthy rabbits of either sex. Rabbits were subjected to forced immobilisation for two hours everyday for consecutive seven days. Blood samples were collected on day 1, 3 and 7 after immobilisation period. Malondialdehyde (MDA), a marker of lipid peroxidation was estimated by TBA assay method. Lipid profile was analysed by multichannel autoanalyser. There was statistically significant increase in MDA, total cholesterol, LDL, VLDL and triglycerides with immobilisation. These findings indicate that stress in the form of forced immobilisation increases lipid peroxidation and alter lipid profile, which may be responsible for pathophysiology of various diseases.

KEY WORDS

Immobilisation stress, Lipid peroxidation, Lipid profile, Rabbits

INTRODUCTION

Stress is an adaptive response that prepares the organism for a threatening situation. It induces strain upon both emotional and physical endurance which has been considered a basic factor in the aetiology of a number of diseases e.g. cardiovascular diseases, cancer, diabetes mellitus, etc (1). Stressful conditions lead to formation of excessive free radicals which are a major internal threat to cellular homeostasis of aerobic organisms (2). Free radicals are formed in human body both in physiological and pathological conditions in cytosol, mitochondria, lysosomes, peroxisomes and plasma membranes (3). These free radicals are extremely reactive and unstable chemical species, which react with proteins, lipids, carbohydrates and nucleic acids in the body (4).

Exposure of lipids in cell membrane to free radicals stimulates the process of lipid peroxidation (1). The products of lipid peroxidation are themselves

reactive species and lead to extensive membrane, organellar and cellular damage (5). The free radical activity and the extent of tissue damage are related quantitatively to the amount of lipid peroxide level in the blood (6). Malondialdehyde (MDA) is one of the end products of lipid peroxidation and extent of lipid peroxidation is measured by estimating MDA levels most frequently. Increased serum level of MDA has been reported in cardiovascular, neurological and other diseases (7, 8). The biochemical feature which has attracted the most sustained and widespread attention in relation to aetiology and prevention of these diseases is serum cholesterol or its fractions like low density lipoproteins (LDL), high density lipoproteins (HDL). Cholesterol and lipoprotein levels correlate well with the risk of cardiovascular diseases (9). Stress in the form of starvation was found to increase lipid peroxidation and alter lipid profile in rabbits (10).

As stress related disease and death mount, it becomes increasingly important to characterise it. Forced immobilisation produces both emotional as well as physical stress (11). Hence, the present work was undertaken to study the effect of stress in the form of forced immobilisation stress on lipid peroxidation and lipid profile in rabbits.

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MATERIALS AND METHODS

Animals

The study was conducted in 25 albino rabbits of either sex weighing between 1-2 kilograms, obtained from central animal house of the institute. The study was approved by Institutional research and ethics committee. The rabbits were provided food and water ad libitum. The rabbits were housed under standard laboratory conditions at ambient temperature with 12 hour day-night cycle. After an initial period of acclimatisation for 4 weeks to laboratory conditions, the rabbits were used for the study.

Experimentation

The rabbits were subjected to forced immobilisation for two hours everyday for consecutive seven days. Their limbs were tied with ropes and were immobilised by restraining them into rabbit restrainers with only their head and neck outside the restrainer (12).

Blood samples of 5 millilitres (ml) from each rabbit were collected from marginal ear vein in a plain test tube under aseptic conditions. Blood samples collected before the rabbits were subjected to immobilisation on day 1 were treated as control. Blood samples were collected at the end of immobilisation period of two hours on day 1, 3 and 7. Blood samples were allowed to clot at room temperature and serum samples were separated by centrifugation at 3000 rpm.

Estimation of MDA was done by Thiobarbituric acid (TBA) assay method using Elico spectrophotometer (13). Serum total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol and triglycerides (TGs) were analysed on multi-channel autoanalyser

(Hitachi-911) using kits from Boehringer Mannheim Ltd.

Statistical Analysis

All data is expressed as mean±S.E.M. All the results were analysed using student's paired t-test. Association between lipid peroxidation (MDA) and lipid profile was analysed using correlation coefficient (r). P<0.05 was considered as statistically significant.

RESULTS

There was significant increase ($p<0.001$) in MDA levels after day 1 (2.82 ± 0.36 $\mu\text{mol/L}$) and day 3 (3.94 ± 0.40 $\mu\text{mol/L}$) of immobilisation as compared to control value (2.01 ± 0.16 $\mu\text{mol/L}$). Serum MDA level became more than double on 7th day (5.05 ± 0.66 $\mu\text{mol/L}$) of immobilisation as compared to control value (2.01 ± 0.16 $\mu\text{mol/L}$).

Table 1 shows the comparison of the serum concentrations of total serum cholesterol, LDL, HDL, VLDL and TGs before and after immobilisation of given duration in rabbits. There was significant increase ($p<0.01$) in total cholesterol levels after day 3 and day 7 of immobilisation as compared to control value and day 1(2h). Serum total cholesterol levels were also significantly increased ($p<0.001$) after day 7 of immobilisation as compared to day 3 of immobilisation.

There was significant increase ($p<0.001$) in LDL levels after day 3 and day 7 of immobilisation as compared to control value and day 1 (2h). Serum LDL levels were also significantly increased ($p<0.001$) after day 7 of immobilisation as compared to day 3 of immobilisation. There was no significant alteration in HDL concentration after immobilisation

Table 1. Lipid profile before (control) and after forced immobilisation of given duration in rabbits (Values represent Mean $\pm$ SEM)

Duration	T. Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	TGs (mg/dl)
Day 1 (0h)	45.32 $\pm$ 3.88	20.68 $\pm$ 2.43	13.76 $\pm$ 1.13	11.00 $\pm$ 0.80	123.56 $\pm$ 11.36
Day 1 (2h)	45.68 $\pm$ 3.45	22.36 $\pm$ 2.08	12.04 $\pm$ 0.72	10.56 $\pm$ 0.56	143.68 $\pm$ 11.35*
Day 3 (2h)	57.12 $\pm$ 5.32*#	29.72 $\pm$ 3.20*#	12.88 $\pm$ 0.90	14.92 $\pm$ 1.73*#	161.48 $\pm$ 12.03*
Day 7 (2h)	72.48 $\pm$ 4.99*#+	41.08 $\pm$ 3.17*#+	12.96 $\pm$ 0.84	17.84 $\pm$ 1.65*#+	191.76 $\pm$ 14.42*#+
*p<0.001 vs day 1 (0h); #p<0.01 vs day 1 (2h); +p<0.001 vs day 3 (2h); n=25					

of given durations. HDL concentration was in the range of 12.04-13.76 mg/dl throughout the study.

There was significant increase ($p < 0.05$) in VLDL levels after day 3 and day 7 of immobilisation as compared to control value and day 1 (2h). Serum VLDL levels were also significantly increased ($p < 0.05$) after day 7 of immobilisation as compared to day 3 of immobilisation. There was significant increase ($p < 0.05$) in TGs levels after day 1 (2h), day 3 and day 7 of immobilisation as compared to control value. Serum TGs levels were also significantly increased ($p < 0.01$) after day 7 of immobilisation as compared to day 1 (2h) and day 3 of immobilisation.

There is positive correlation of increased MDA with increase in total cholesterol (r value +0.96), LDL ($r + 0.97$), VLDL ($r + 0.83$) and TGs ($r + 0.99$) and this increase is statistically significant. Correlation of MDA with HDL is negative ($r - 0.21$).

DISCUSSION

The findings of this study reveal significant increase in serum MDA levels, total cholesterol, LDL, VLDL and TGs after immobilisation stress of different duration in rabbits. Increased level of MDA is also reported in other stress models of rabbits like starvation stress and ischaemic limb injury in rabbits (10, 14). Increased MDA levels depict increased lipid peroxidation which may be because of excessive production of free radicals after immobilisation stress. The increase is duration dependant. The sharp increase in MDA on day 1 (2h) may be because of exposure of rabbits to immobilisation stress for the first time.

The increase in total serum cholesterol concentration and LDL is in agreement with previous studies (10, 15, 16). These results support the hypothesis that cholesterol stored in the lipid droplets of the adipose tissue cells is released into plasma and is the chief source of the hypercholesterolemia observed during stress. Another cause for hypercholesterolemia during stress may be continued biosynthesis concomitant with decreased or completely absent intestinal excretion (17). The increase in LDL levels is duration dependant in this study. Increased concentrations of LDL were reported in male medical students near exams as compared to pre exam levels (16). There is also increase in VLDL levels in this study. VLDL is reported to be increased in different hyperlipoproteinemias (type II, III, IV and V) and may be a contributing factor in atherosclerosis (18). No previous study has reported effect of immobilisation stress on VLDL

concentration. In the present study, immobilisation stress also caused significant duration dependant increase in TGs levels. The increase in TGs levels may be because of release of TGs from storage sites for the formation of glucocorticoids in response to immobilisation stress (12).

In the present study it is observed that HDL levels are almost constant after stress. Previous studies have reported either no change or decrease in HDL levels (16). This may be due to different experimental preparation and individual differences in susceptibility to stress.

The lipoprotein fractions are more predictive of developing coronary artery disease instead of total cholesterol. LDL is well recognised as a risk factor and HDL as a protective factor against atherosclerosis (19). In addition, it has been shown that HDL/total cholesterol ratio is one of the most powerful predictors of risk of developing CAD (12). HDL/total cholesterol ratio has decreased in this study. Immunocyto-chemical analysis has revealed the presence of protein modified by MDA, which convert LDL, the major carrier of plasma cholesterol, to an abnormal form. Receptor-mediated clearance of this altered LDL produces cholesteryl ester deposition in macrophage-derived foam cells of atheromas. These findings provide direct evidence for the existence of protein modified by a physiological product of lipid peroxidation within arterial lesions (20). Plasma samples from hypercholesterolemic rabbits and from several human subjects have been found to contain antibodies that were found to react with peroxidised LDL, suggesting that peroxidation does indeed take place in vivo (20,21). Correlation of lipid profile and lipid peroxidation after immobilisation is positive which indicates that increased lipid peroxidation is associated with increased total cholesterol and LDL, the known major risk factors for atherosclerosis and other lipid peroxidation induced diseases. Increased VLDL concentration may also have some role in atherogenesis (18). Negative correlation of MDA with HDL suggests that increased lipid peroxidation is associated with decreased HDL concentration. Decreased HDL levels may be a contributing factor in atherogenesis. In this study, increased lipid peroxidation, total cholesterol, LDL, VLDL and TGs may predict increased susceptibility of the animals to atherosclerosis as a result of immobilisation stress.

These findings suggest that there is stress induced accelerated lipid peroxidation (increased MDA) and alterations in lipid profile affecting serum total cholesterol, LDL, VLDL and triglycerides.

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