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Research report

Effect of chronic unpredictable stress on short term dietary restriction and its modulation by multivitamin-mineral supplementation ☆

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ABSTRACT

Dietary restriction (DR) lowers steady-state levels of oxidative stress and alters behavioral, physiological and biochemical responses in mammals. However, various factors effect its application in humans like socio-cultural, appetite and the daily life stress. Physiological and psychological stress owing to fast-paced lifestyles, translates into oxidative stress. In this work, the role of chronic unpredictable stress (CUS) on the effects of short term DR in mice in terms of biochemical and oxidative stress parameters was investigated. Further, the modulatory role of multivitamin-mineral supplement (MVM) on CUS and DR induced biochemical changes was studied to delineate the role of micronutrient supplementation. DR treatment increased the antioxidant status in the circulation and liver of mice but in the presence of chronic stressors there was a significant shift towards the pro-oxidant state. A decrease was found in the activities of antioxidant enzymes superoxide dismutase, catalase, and glutathione-S-transferase and glutathione reductase in the rats exposed to CUS with DR (CUS + DR), with an increased malondialdehyde and a decreased glutathione (GSH) levels as compared to the controls. Liver function enzymes–glutamate oxaloacetate transaminase and glutamate pyruvate transaminase were increased and a significant DNA damage was observed. Oral MVM supplement significantly improved this oxidative deterioration. Hence, MVM supplementation appears to potentially offer an effective intervention in the DR regimen to combat daily life physical and mental stress.

Introduction

Dietary Restriction (DR) is a term commonly used to describe a 20–40% reduction in calorie intake, although it can also refer to more or less severe restrictions to reduced daily intake of particular components of the diet, such as amino acids, proteins or fats (Fontana, Partridge, & Longo, 2010). The physiological effects of dietary or caloric restriction (DR/CR) on life span, disease and aging have been reported extensively in rodent models (Colman et al., 2009; Ribeiro et al., 2012). CR, compared with *ad libitum* (AL) feeding, is shown to increase the life span of laboratory mice and rats (Fishbein, 1991). The health benefits of CR are mediated through a harmony of redox homeostasis, mitochondrial function, inflammation, apoptosis and autophagy (Han & Ren, 2010). CR regimen in rodents results in many physiological changes, including reductions in body weight, temperature, blood glucose, and insulin levels (Sohal & Weindruch, 1996). CR decreases metabolic rate, oxidative damage and cell proliferation, enhances autophagy and DNA repair processes (Fontana & Klein, 2007). Several studies have demonstrated that CR reduces damage to proteins, lipids, and DNA by diminishing the steady-state concentrations of the products of oxidative damage and decreases the *in vitro* susceptibility of different tissues to acute oxidative stress and the associated decline in

Abbreviations: AL, *ad libitum*; CAT, catalase; CR, caloric restriction; CUS, chronic unpredictable stress; DR, dietary restriction; GC, glucocorticoid; GOT, glutamate Oxaloacetate Transaminase; GPT, glutamate pyruvate transaminase; GR, glutathione reductase; GSH, reduced glutathione; GST, glutathione S-transferase; MDA, malondialdehyde; MVM, multivitamin-mineral; ROS, reactive oxygen species; SOD, superoxide dismutase.

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organ function (Sohal, Agarwal, Candas, Forster, & Lal, 1994). The antioxidant effect of CR may involve reduced production of mitochondrial superoxide anion, H_2O_2, with decreased free radical induced DNA damage and a concomitant increase in antioxidative defenses, though activities of individual antioxidative enzymes do not follow a consistent pattern in response to CR (Naziroglu & Brandsch, 2006; Sohal, Agarwal, et al., 1994; Sohal, Ku, Agarwal, Forster, & Lal, 1994).

Practicing long term DR is not possible in humans who often follow short term or transient DR. This tempted us to investigate the effects of chronic stress on animals simultaneously kept on DR regimen, to probe if the stress exposed animals exhibit physiological response similar to the one practicing only DR. Our study investigated the effects of short term DR feeding regimen (21 days) on biochemical parameters and DNA integrity. We speculated that oxidative stress response to DR can be varied when the animal is exposed to chronic stress which can lead to a shift either towards or away from the pro-oxidant status (Watson et al., 2003). Further, we report that MVM supplementation during DR has modulatory role on this oxidative shift.

Materials and methods

Experimental animals

Four to five week old male Swiss albino mice (30 ± 5 g) were used in the present study. The animals were maintained in controlled atmosphere of 12 h dark/light except Groups II, IV, and V during day 5 of each week when the dark/light cycle was altered according to the protocol schedule, 22 ± 2 °C temperature and 50–60% humidity and sacrificed by cervical dislocation with minimal suffering following rules laid down by Animal Welfare Committee of the A.M. University, Aligarh as per the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, 714/02/a/CPCSEA) norms.

Chronic Unpredictable Stress (CUS)

The CUS protocol is a modification of published procedures (Oritz, Fitzgerald, Lane, Terwilliger, & Nestler, 1996). The CUS sequence consisted of different types of stressors presented randomly, over a period of 21 days as outlined in Table 1. Specific details of the CUS procedure are as follows: for restraint stress, mice were placed individually in body sized wire mesh cages attached to wooden boards, with no movement allowed. Wet bedding was carried out by filling 300 ml tap water in home cage. Forced swim and cold forced swim was accomplished by placing the mice in a cylindrical tank (50 cm height × 20 cm diameter) filled with water to a 20 cm depth at 25 or 4 °C, respectively. Crowding was done by placing an iron divider in the cage to provide minimum space for housing. Lastly illumination was attained by placing an illuminated tube light on the cages for overnight. After each stressor, animals were kept in a recovery room for 1–2 h, following which they were placed in clean cages with fresh bedding and returned to the housing facility.

Table 1
Weekly CUS protocol.

Day	Stress type and schedule
1	1000 h, restraint, 3 h
2	1100 h, wet bedding (25 °C), 2 h
3	1500 h, forced swim (25 °C), 30 min
4	1300 h, crowding, 2 h
5	1900 h, lights on, overnight
6	0900 h, cold forced swim (4 °C), 15 min; 2200 h, crowding, overnight
7	1000 h, restraint, 2 h 1900 h, food deprivation, overnight

Experimental design

Mice were randomly divided into five groups of 16 mice each and a treatment schedule of 21 days was adopted (Table 1):

Group I (Ad libitum or AL): Mice were allowed to feed on mice chow diet freely without any daily feed intake restriction.
Group II (CUS): Mice were subjected to CUS paradigm for 21 consecutive days.
Group III (DR): Mice were fed on mice chow diet with daily feed intake restricted to 75% of the control group. Ration levels for this group was based on the previous 24-h average food consumption of the AL group.
Group IV (CUS + DR): Mice were subjected to dietary restriction along with simultaneous exposure to CUS.
Group V (CUS + DR + MVM): Mice were subjected to CUS paradigm and DR regimen followed by supplementation with a multivitamin-mineral (10 μg/gram body weight/day, p.o.) for 21 consecutive days. The dose of MVM selected for feeding mice is based on the recommendation that a typical healthy individual will take one 490 mg Galoxy MVM tablet per day.

The antioxidant efficacy of MVM alone and in context with stress has already been mentioned in our earlier study (Hasan et al., 2011).

After 21 days of CUS paradigm, animals from all the groups were sacrificed by cervical decapitation and blood and liver samples of eight mice from each group were collected for biochemical estimations. Blood was collected in heparinized tubes and plasma was obtained by centrifugation at 1500*g* for 10 min at 4 °C. Liver tissues were rinsed with normal saline. A weighed portion of the tissues were homogenized in chilled 0.1 M phosphate buffer pH 7.4 using glass Teflon homogenizer and volume was adjusted to give 10% w/v homogenate. The homogenates were centrifuged at 3600*g* for 20 min at 4 °C to remove cellular debris and the supernatant was used for further studies. All the experimental protocols adhered to the guidelines of the Institutional Ethical Committee of the A.M. University (CPCSEA-714/02/a). Remaining eight mice from each group were sacrificed and their blood and liver tissues were subjected to DNA damage analysis by alkaline comet assay.

Chemicals

Multivitamin-mineral supplement (Galoxy 490 mg) was purchased from Roots Life Sciences Pvt. Ltd. India; vitamin E (Evion 200 mg) was obtained from Merck, India. Vitamin C (Celin 500 mg) was purchased from GlaxoSmithKline Pharmaceuticals Ltd., India. NADPH; oxidized and reduced glutathione; 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent); 1-chloro-2,4-dinitrobenzene (CDNB) and Pyrogallol were acquired from SRL India. All other chemicals were of analytical grade.

Biochemical estimations

All the biochemical estimations were performed both in the plasma (circulation) and liver tissues.

SOD activity was assayed by monitoring the inhibition of auto-oxidation of pyrogallol (0.05 M tris succinate buffer, pH 8.2) at 420 nm. One enzyme unit is defined as the amount of enzyme required to cause 50% inhibition of the rate of pyrogallol auto-oxidation (Marklund & Marklund, 1974). CAT activity was measured in 0.05 M phosphate buffer (pH 7.0) by following the decrease in absorbance at 240 nm due to decomposition of 30 mM hydrogen