Original Research

Nrf2 induction alleviates metabolic disorder and systemic inflammatory response in rats under a round-the-clock lighting and high-carbohydrate–lipid diet

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Abstract

Background and aims: The aim of this work is to study the effect of Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) inductor dimethyl fumarate (DMF) on the metabolic disorder and systemic inflammatory response in rats exposed to round-the-clock lighting (RCL) and kept on the high-calorie carbohydrate–lipid diet (HCLD). **Materials and methods:** The experiment was performed on 21 Wistar white male rats. Control rats (Group 1) were fed standard control chow and kept on 12/12 hours light / dark cycle. Rats of the Group 2 were kept on a HCLD for 8 weeks and exposed to RCL, thus simulating metabolic syndrome development. The animals of Group 3 received DMF in 10% dimethyl sulfoxide solution at a dose of 15 mg/kg intraperitoneally 3 times a week starting on the 30th day of the experiment. **Results:** DMF administered against the RCL and HCLD background lowered the serum glucose, insulin, cholesterol of very-low-density lipoproteins and triglycerides levels and elevated the cholesterol of high-density lipoproteins concentration compared with the findings of Group 2. Administering DMF under RCL and HCLD reduced serum tumor necrosis factor-alpha, C-reactive protein, and blood thiobarbituric acid-reacting compounds concentration compared to the results in Group 2. **Conclusions:** The induction of the Nrf2 signaling system can be regarded as a potential target in the experimental therapy of metabolic syndrome, triggered by the disturbance of light / dark cycle and Western diet.

Keywords: transcriptional factor Nrf2, metabolic syndrome, light-dark cycle, Western-style diet, systemic inflammation.

Background and aims

A large number of existing experimental and clinical studies have shown that disruption of the natural light–dark cycle accompanied by hypomelatoninemia results in the carbohydrate and lipid metabolism shifts, systemic inflammatory response, hypertension, endothelial dysfunction, and oxidative stress [1, 2]. All these disorders are known as components of metabolic syndrome (MS). For instance, there has been found out employees of long-distance train crews are at a high-risk to develop insulin resistance (IR) and other manifestations of this syndrome [3].

Recent reports have demonstrated that diet and nutrients can modulate the fluctuations in melatonin, but the effect of these factors is less important than the circadian cycle [4]. The combined action of round-the-clock lighting

(RCL) and prolonged keeping on the high-calorie carbohydrate–lipid diet (HCLD, 20% fructose solution and proper ratio) in experiments on rats lead to more pronounced metabolic disorders (hypomelatoninemia, hyperinsulinemia, dyslipoproteinemia, hypo-α-lipoproteinemia, hypertrialcylglycerolemia, and increased visceral fat mass) than under the separate actions of the factors mentioned above [5]. Nevertheless, melatonin administered under these conditions can lessen these signs to some extent without affecting the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) [6]. The obtained results have shown that the melatonin level having been restored is insufficient for correcting experimental metabolic disorders associated with long-term activation of some redox-sensitive transcription factors, in particular, NF-κB [7]. This can result from both an excess in the dietary carbohydrates and fats ("Western-style diet") and changes in the expression of inducible genes of central and peripheral circadian oscillators in RCL laboratory animals [8].

Our previous investigation has revealed that the introduction of the NF-κB inhibitor ammonium pyrrolidine dithiocarbamate to rats exposed to RCL and kept on HCLD lowers glucose serum concentration, restricts hyperinsulinemia and IR, alleviates the manifestations of dyslipoproteinemia, hypo-α-lipoproteinemia, and hypertriacylglycerolemia, as well as inhibits the development of systemic inflammatory response (SIR) [9]. However, this NF-κB inhibitor demonstrates a number of adverse effects, and genotoxicity is among them [10].

Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2)-related signaling system is considered antagonistic to NF-κB-signaling. Nrf2 serves as a regulator of cellular resistance to pro-oxidants, it regulates basal and induced expression of many genes, which contain cis-regulatory enhancers with the nucleotide sequence 5′-puGTGACNNNGC-3' (N represents any nucleotide), known as Antioxidant Response Element (ARE), in their promoter regions [11].

According to the experimental data obtained from Nrf2 knockout laboratory animals, Nrf2 double knockout animals, Keap1 gene-knockout animals [12], and laboratory animals, who, by contrast, received Nrf2 / ARE system inductors, this system activates gene expression of a series of antioxidative and cytoprotective proteins including heme oxygenase-1, NAD(P)H quinone dehydrogenase 1, c-glutamyl-cysteine synthetase, glutathione peroxidase 1, glutathionine S-transferase, glutathione reductase, and superoxide dismutase [13].

The use of bioflavonoids (quercetin, epigallocatechin-3-gallate), capable of inducing Nrf2 / ARE system, considerably reduces the level of pro-inflammatory cytokines, and, particularly, interleukins 1 and 6, tumor necrosis factor-alpha (TNF-α), and acute-phase proteins in blood serum, as well as nitro-oxidative markers in different organs under SIR modelled by intraperitoneal lipopolysaccharide (LPS) injection and traumatic brain injury [14–16].

At the same time, these bioflavonoids, impacting Nrf2 / ARE system, also have an effect on other transcriptional factors (NF-κB, AP-1, etc.), and can directly influence free radicals and different enzymes (lipoxygenase, cyclooxygenase, xanthine oxidase, cytochrome P450-containing monooxygenases) [17]. This adds to the complexity of defining the role of the Nrf2-signalling pathway in experimental therapy of metabolic syndrome, nitro-oxidative stress, and SIR.

Therefore, the aim of this study is to investigate the effect of Nrf2 inductor dimethyl fumarate on the metabolic disorder and systemic inflammatory response in rats exposed to round-the-clock lighting and kept on a diet rich in carbohydrates and lipids.

Materials and methods

Animals data and ethics statement

The experiment was performed on 21 Wistar white male rats weighing 215–255 g kept under standard vivarium conditions (air temperature: +22±2°C, air humidity: 30–60%). Control rats (Group 1, n=7) were fed standard chow and kept on 12/12 hours light / dark cycle. Rats of Group 2 (n=7) were kept on an HCLD for 8 weeks and exposed to RCL, thus simulating MS development. The animals of Group 3 (n=7) received

dimethyl fumarate (DMF, "Sigma-Aldrich, Inc.", USA) in 10% dimethyl sulfoxide solution at a dose of 15 mg/kg [18] intraperitoneally three times a week starting on the 30th day of the experiment.

Male rats of the first two groups, instead of receiving dimethyl fumarate intraperitoneally, were given a "placebo", 1 ml of 10% dimethyl sulfoxide solution.

The experiment complied with the requirements of the European Convention for the protection of vertebrate animals used for research and other scientific purposes (Strasbourg, 1986) and the European Union Directive 2010/10/63 EU on animal experiments. The Commission on Bioethics of Petro Mohyla Black Sea National University did not find any violations of moral and ethical norms during this study. The rats were decapitated with ethereal anesthesia.

Experimental model of metabolic syndrome

To simulate MS, the rats were being kept on HCLD for 2 months: the animals received a 20% aqueous fructose solution for drinking and a diet containing refined wheat flour (45%), skimmed milk powder (20%), starch (10%), table margarine with fat content 72–82% (20%), peroxidized sunflower oil (4%), sodium chloride (1%). On the 30th day of the experiment, the rats were exposed to RCL with an intensity of 1500 lx over the next 30 days [19].

Biochemical and enzyme-linked immunosorbent assays

Glucose level and the lipid profile in the blood serum were determined by using standard laboratory reagents, reagent kits ("Philisit-Diagnostics", Ukraine), and a spectrophotometer Ulab 101 (China). Glucose level was measured by the glucose oxidase method.

The total cholesterol (CH), high-density lipoproteins (HDL) cholesterol, and triglycerides (TGs) were measured by the enzymatic method according to the concentration of quinonymine formed, which is proportional to these lipids.

The cholesterol of low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) were calculated from total CH, HDL cholesterol and fasting TGs levels using the Friedewald equation: LDL cholesterol (mmol/l) = (total CH – HDL cholesterol – TGs) / 2.2; VLDL cholesterol (mmol/l) = TGs / 2.2 [20].

The concentration of insulin, TNF-α, and C-reactive protein (CRP) in the blood serum were measured by the enzyme immunoassay method using Rat ELISA Kits (MyBioSource.com, USA).

HOMA-IR, a method used to quantify IR, was calculated by the following equation: HOMA-IR = fasting glucose (mmol/l) × fasting insulin (µU/ml) / 22.5 [21].

The level of lipid peroxidation (LPO) in the blood was evaluated by the formation of a stained trimethine complex during the reaction of thiobarbituric acid (TBA) and TBA-active products before and after 1.5-hour incubation of homogenate in the pro-oxidant iron-ascorbate buffer solution [22]. The activity of the antioxidant system was assessed by an increase in the concentration of TBA active compounds, secondary LPO products, during 1.5-hour incubation in pro-oxidant iron-ascorbate buffer solution [22].

Statistical analysis

The findings obtained were statistically processed using the Microsoft Office Excel software package with the Real Statistics 2019 extension using the Shapiro-Wilk test to check the normality of variances. Basic statistics, such as arithmetic mean (M) and standard error of the mean (m) were calculated. Results in the tables have been provided as M±m. As far as all samples had normal distribution, we applied the ANOVA parametric analysis of variance, followed by a pairwise comparison of groups by Student's t-test for independent samples and Tukey's HSD (Honestly Significant Difference) analysis. The Dunn–Šidák correction was used to avoid the phenomenon of multiple comparisons. The differences between arithmetic means were considered significant at $p < 0.05$.

Results

The rats exposed to RCL with an intensity of 1500 l× and kept on HCLD demonstrated considerable deterioration of carbohydrate metabolism parameters (Table 1). Glucose concentration exceeded the respective values in the control rats 1.4 times. The concentration of insulin and HOMA-IR was higher at 3.79 and 3.65 times, respectively, compared with Group 1.

DMF administered against the RCL and HCLD background lowered serum glucose concentration, which was inferior by 14.2% to the data of Group 2. Under this condition, the serum insulin concentration and HOMA-IR declined 1.71 and 1.74 times, respectively, compared with the findings of Group 2.

But the rats, when being exposed to RCL with an intensity of 1500 lx and received HCLD, demonstrated a considerable fall in HDL-CH concentration, 1.64 times compared with the respective parameter in the control rats. The concentration of VLDL-CH and TGs, on the contrary, increased significantly and exceeded 3.31 and 3.23 times, respectively, the values of Group 1.

DMF administered against the background of RCL and HCLD elevated the HDL-CH concentration, which was 2.21-time higher than the results in Group 2. Under these conditions, VLDL-CH and TGs concentrations subside by 1.68 times for both compared with the findings in Group 2.

The study of blood serum under experimental MS revealed the signs of SIR (Table 3).

Table 1: Effect of dimethyl fumarate on carbohydrate metabolism parameters in the blood serum under experimental metabolic syndrome.

Parameters	Control rats (only "placebo" -10% dimethyl sulfoxide solution), n=7	RCL+HCLD	
		+10% dimethyl sulfoxide solution ("placebo"), n=7	+Dimethyl fumarate in 10% dimethyl sulfoxide solution, n=7
Glucose, mmol/l	4.94±0.24	6.89±0.25*	4.24±0.19*,**
Insulin, µU/ml	1.45±0.23	5.5±0.22*	1.62±0.13**
HOMA-IR	0.32±0.06	1.17±0.04*	0.30±0.02**

Note: *p<0.05 compared to findings in the control group; **p<0.05 compared to findings in the rats of Group 2. HCLD – High-calorie carbohydrate-lipid diet; HOMA-IR – Homeostasis Model Assessment of Insulin Resistance; RCL – Round-the-clock lighting.

Table 2: Effect of dimethyl fumarate on lipid profile in the blood serum under experimental metabolic syndrome.

Parameters	Control rats (only "placebo" -10% dimethyl sulfoxide solution), n=7	RCL+HCLD	
		+10% dimethyl sulfoxide solution ("placebo"), n=7	+Dimethyl fumarate in 10% dimethyl sulfoxide solution, n=7
Total CH, mmol/l	2.39±0.29	2.62±0.30	2.36±0.23
HDL-CH, mmol/l	0.63±0.04	0.23±0.02*	0.51±0.03*,**
LDL-CH, mmol/l	1.47±0.29	1.43±0.29	1.54±0.24
VLDL-CH, mmol/l	0.29±0.02	0.96±0.04*	0.31±0.03*,**
TGs, mmol/l	0.65±0.05	2.10±0.10*	0.68±0.07**

Note: *p<0.05 compared to findings in the control group; **p<0.05 compared to findings in the rats of Group 2. RCL – Round-the-clock lighting
CH – Cholesterol; HCLD – High-calorie carbohydrate-lipid diet; HDL – High-density; LDL – Low density lipoproteins; TGs – Triglycerides VLDL – Very low density lipoproteins.

Table 3: Effect of dimethyl fumarate on TNF-α and CRP levels in the blood serum under experimental metabolic syndrome.

Parameters	Control rats (only "placebo" -10% dimethyl sulfoxide solution), n=7	RCL+HCLD	
		+10% dimethyl sulfoxide solution ("placebo"), n=7	+Dimethyl fumarate in 10% dimethyl sulfoxide solution, n=7
TNF-α, pg/ml	34.02±2.00	109.75±6.01*	34.87±2.40**
CRP, ng/ml	4.08±0.12	12.75±0.31*	4.08±0.10**

Note: *p<0.05 compared to findings in the control group; **p<0.05 compared to findings in the rats of Group 2. CRP – C-reactive protein, HCLD – High-calorie carbohydrate-lipid diet; RCL – Round-the-clock lighting; TNF-α – Tumour necrosis factor-alpha.

Table 4: Effect of dimethyl fumarate on the secondary LPO products in the blood under experimental metabolic syndrome.

Parameters	Control rats (only "placebo" -10% dimethyl sulfoxide solution), n=7	RCL+HCLD	
		+10% dimethyl sulfoxide solution ("placebo"), n=7	+Dimethyl fumarate in 10% dimethyl sulfoxide solution, n=7
TBA-reacting compounds, μmol/kg			
Before incubation	11.19±0.90	23.04±0.55*	11.57±0.46**
After incubation	25.41±1.98	48.32±2.10*	26.89±1.52**
Increment over incubation time	14.22±2.36	25.28±1.92	15.32±1.64**

Note: *p<0.05 compared to findings in the control group; **p<0.05 compared to findings in the rats of Group 2. HCLD – High-calorie carbohydrate-lipid diet; LPO – Lipid peroxidation; RCL – Round-the-clock lighting; TBA – Tiobarbituric acid.

Concentrations of TNF-α, a pro-inflammatory cytokine, CRP, and acute phase reactant under the conditions of RCL and HCLD exceeded the parameters in the control rats by 3.22 and 3.12 times.

DMF administered during RCL and HCLD combination lowered serum TNF-α and CRP concentration by 68.2% and 68.0%, respectively, compared to the results in Group 2.

Experimental MS was manifested by the changes in blood parameters of LPO and antioxidant provision in the rats (Table 4). The concentration of TBA-reacting compounds, secondary LPO products under RCL and HCLD rose 2.05 times (before incubation) and 1.9 times (after incubation) compared to the respective values in the control rats. The increment of the TBA-reacting compounds for 1.5-hour incubation in a pro-oxidant iron-ascorbate buffer solution 1.78 times exceeded the findings of Group 1 that pointed considerable decrease in antioxidant reserve in the blood of rats.

Administering DMF under RCL and HCLD reduced the concentration of TBA-reacting compounds in the blood 1.5 times (before incubation) and 1.44 times (after incubation) compared to the respective values in Group 2. The increment of the TBA-reacting compounds over incubation in pro-oxidant buffer solution by 39.4% was inferior to the values of Group 1 which evidenced the increase in blood antioxidant reserve under the DMF administration.

Discussion

The results obtained confirm that RCL and HCLD, which included a 20% aqueous

fructose solution and special ration, cause carbohydrate and lipid metabolic disorders typical of metabolic syndrome. In our previous study, we have shown that the combined effect of RCL and HCLD can make MS signs such as hyperinsulinemia, dyslipoproteinemia, hypo-α-lipoproteinemia, hypertrialcylglycerolemia, and visceral fat more pronounced in comparison with the animals kept on the isolated diet only [5]. It has been also found that these conditions significantly reduce the melatonin concentration in the blood serum compared with the results obtained in the animals exposed to the RCL only.

It is known that TGs and metabolites of non-esterified fatty acids – CoA-activated fatty acids, diacylglycerol, and ceramides – can cause severe IR. This is due to impaired insulin signaling, for example, due to serine phosphorylation of insulin receptor substrate proteins IRS1 [23].

The question about the common link of the MS pathogenesis that would be able to integrate all its components is being widely discussed now. The assumption that prolonged activation of transcription factors NF-κB, AP-1, STAT-3, or impairment of the induction of antagonistic transcription factors (Nrf2, PPAR) can serve as such a universal mechanism, has been underpinned theoretically and experimentally [24–27]. Recent studies have demonstrated that the Nrf2 signaling pathway explains some of the therapeutic and biological effects of melatonin [28].

In fact, when reproducing the MS model, in which HCLD aggravates RCL-induced hypomelatoninemia [5] in this investigation, in addition to carbohydrate and lipid metabolic disorders, we observed the SIR development (elevation of TNF-α and CRP levels) and oxidative stress against the background of the blood reduced antioxidant reserve (increase in TBA-reacting compounds and their increment for 1.5-hour incubation in pro-oxidant iron-ascorbate buffer solution).

The use of DMF, the Nrf2 inductor, improves carbohydrate and lipid metabolism in experimental MS modeled by RCL and HCLD, and in particular, lowers the serum glucose and insulin concentration that is accompanied by the decrease in HOMA-IR, restricts the increase in VLDL-CH and TG levels in the blood serum under considerable HDL-CH growth. Under these conditions the levels of pro-inflammatory cytokines and acute-phase reactants (TNF-α, and CRP) decline, as well as secondary LPO products (TBA-reacting compounds).

An important role of Nrf2 / ARE system is determined by the fact that its activity determines the condition of NF-κB- and AP-1-associated signaling pathways. In response to LPS, Nrf2 knockdown significantly increases the NF-κB activity with NF-κB-dependent gene transcription that shows the Nrf2 capability to impede NF-κB activity [29]. It has been demonstrated that Nrf2 inducers (phenethyl isothiocyanate, sulforaphane, and curcumin) suppress the NF-κB activation [30]. The administration of phenethyl isothiocyanate and sulforaphane inhibits phosphorylation at the site of IκB kinase complex (IKK) / IκB (inhibitor of NF-κB) protein with subsequent p65 subunit nuclear translocation, thus impairing NF-κB-dependent signaling pathway [31]. There has been reported about the possibility to reduce the activity of IKKβ [32] and caspase-mediated proteolysis of NF-κB/p65 by applying Nrf2 inducers [33].

Except for restraining adverse consequences of NF-κB-associated signaling pathways, the LPO restriction and increase in the blood antioxidant reserve can be related to Nrf2 / ARE-dependent activating of the gene expression of antioxidative proteins such as glutathione peroxidase 1, glutathione reductase, superoxide dismutase, etc. [13].

Conclusions

The administration of DMF, an Nrf2 inductor, under experimental metabolic syndrome, modeled by carbohydrate–lipid diet and round-the-clock lighting, reduces serum glucose concentration, restricts hyperinsulinemia and insulin resistance, lessens the signs of dyslipoproteinemia, hypo-α-lipoproteinemia, and hypertrialcylglycerolemia, inhibits systemic inflammatory response, lipid peroxidation, and improves the blood antioxidant reserve. The induction of the Nrf2 signaling system can be regarded as a potential target in the experimental

therapy of metabolic syndrome, triggered by the disturbance of the light / dark cycle and the Western diet.

Conflict of Interest

The authors declare no conflict of interest.

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