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Quercetin ameliorates chronic unpredicted stress-induced behavioral dysfunction in male Swiss albino mice by modulating hippocampal insulin signaling pathway

Vineet Mehta[a], Tiratha Raj Singh[a], Malairaman Udayabanu[a,*]

[a] Department of Biotechnology, Bioinformatics, and Pharmacy, Jaypee University of Information Technology, Waknaghat, India

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ABSTRACT

Chronic stress is associated with impaired neurogenesis, neurodegeneration and behavioral dysfunction, whereas the mechanism underlying stress-mediated neurological complications is still not clear. In the present study, we aimed to investigate whether chronic unpredicted stress (CUS) mediated neurological alterations are associated with impaired hippocampal insulin signaling or not, and studied the effect of quercetin in this scenario. Male Swiss albino mice were subjected to 21 day CUS, during which 30 mg/kg quercetin treatment was given orally. After 21 days, behavioral functions were evaluated in terms of locomotor activity (Actophotometer), muscle coordination (Rota-rod), depression (Tail Suspension Test (TST), Forced Swim Test (FST)) and memory performance (Passive-avoidance step-down task (PASD)). Further, hippocampal insulin signaling was evaluated in terms of protein expression of insulin, insulin receptor (IR) and glucose transporter 4 (GLUT-4) and neurogenesis was evaluated in terms of doublecortin (DCX) expression. 21 day CUS significantly impaired locomotion and had no effect on muscle coordination. Stressed animals were depressed and showed markedly impaired memory functions. Quercetin treatment significantly improvement stress-mediated behavior dysfunction as indicated by improved locomotion, lesser immobility time and greater frequency of upward turning in TST and FST and increased transfer latency on the day 2 (short-term memory) and day 5 (long-term memory) in PASD test. We observed significantly higher IR expression and significantly lower GLUT-4 expression in the hippocampus of stressed animals, despite of nonsignificant difference in insulin levels. Further, chronic stress impaired hippocampal neurogenesis, as indicated by the significantly reduced levels of hippocampal DCX expression. Quercetin treatment significantly lowered insulin and IR expression and significantly enhanced GLUT-4 and DCX expression in the hippocampus, when compared to CUS. In conclusion, quercetin treatment efficiently alleviated stress mediated behavioral dysfunction by modulating hippocampal insulin signaling and neurogenesis.

1. Introduction

Stress response is an essential defense mechanism of our body, which enables us to deal with the daily life stressors [1,2]. However, chronic stress is highly deleterious process, which initiates several degenerative processes throughout the body, especially in the brain, which is highly vulnerable to stress mediated damage. In the CNS, chronic stress is associated with impaired synaptic plasticity [3,4], reduced hippocampal volume [2] and reduced dendritic complexity [5], besides being associated with neurodegeneration [6], depression and impaired learning and memory functioning [7,8], which compromises healthy state of living.

Mechanisms responsible for the development and progression of chronic stress-mediated neurological complications are poorly understood. Previous studies suggest that chronic stress is associated with enhanced oxidative and inflammatory stress [8], neurodegeneration, glucose intolerance, insulin resistance and impaired insulin signaling in the brain, which plays a part in the development of these complications [9,10]. Brain utilizes large proportion of the body's glucose and regulates the energy homeostasis throughout the body, and therefore, a continuous and uninterrupted glucose supply is essential for the proper functioning of neurons. Unlike peripheral system, role of insulin and insulin signaling in the brain energy homeostasis is debatable. Evidence suggest that insulin signaling is essential for glucose utilization and metabolism in the brain [11,12], while it is also reported that this process is independent of the insulin actions [13,14]. Interestingly,

* Corresponding author at: Department of Biotechnology, Bioinformatics, and Pharmacy, Jaypee University of Information Technology, Waknaghat, Solan 173234, India.
E-mail address: udayabanu.m/Juit@juit.ac.in (M. Udayabanu).

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insulin receptors (IR) and glucose transporters (GLUT) are abundantly distributed in the cortex, hippocampus and hypothalamus regions of the brain, suggesting that energy homeostasis in neurons may not be independent of insulin and insulin signaling pathway [15]. It is now evident that insulin and insulin signaling is having neuromodulatory potential and intact insulin signaling in the brain is essential for proper behavioral functioning and maintaining synaptic plasticity and neurogenesis [15]. Impaired insulin signaling has been associated with impaired neuronal plasticity, enhanced neuronal oxidative stress [16], neurodegeneration [17], cognitive decline [18] and neurodegenerative disorders such as Alzheimer's disease [17]. Further, glucose utilization in the brain depends primarily on the levels of GLUT-4, which are controlled directly by the insulin-signaling pathway. Recent reports suggest that hippocampal GLUT-4 expression is essential for normal learning and memory functions [10,19]. Besides, chronic stress disrupts hippocampal neurogenesis, which further impair neuronal functioning [20].

Chronic unpredicted stress (CUS) model in mice is widely used to investigate the neurological complications and physiological alterations associated with chronic stress. CUS is associated with the development of glucose intolerance, insulin signaling dysfunction and marked behavioral alterations, which includes anxiety, depression-like behavior, and cognitive dysfunction [8,10,21]. Previously, we have demonstrated that treating stressed animals with hydroalcoholic leaf extract of *Urtica dioica* efficiently improve CUS mediated behavioral dysfunction by modulating hippocampal insulin signaling pathway. We further observed that quercetin is present in high amount in *U. dioica* extract, which may be responsible for its neuromodulatory potential [8,21]. We also demonstrated that quercetin is having neuroprotective potential and is capable of upregulating neuronal GLUT-4 expression through in-vitro assays [22]. Treating stressed animals with quercetin alleviated behavioral alterations and improved neuronal morphology, which were associated with reduced hippocampal oxidative and inflammatory stress [8]. Quercetin is a natural antioxidant and is known to improve neuronal survival and functioning [23], reduce neuronal oxidative stress [8], insulin resistance [24] and blood cortisol levels [25], besides having potential to attenuate learning and memory dysfunction, anxiety and depressive like behavior in rodents [26,27]. In this follow-up study, we aimed to explore the effect of CUS on insulin signaling and neurogenesis in the hippocampus region of the brain and its association with memory dysfunction and depression in mice, and explored the effect of quercetin in this scenario.

2. Material and methods

2.1. Animals

Male Swiss albino mice, weighing 20–25 g, were used in the entire experimentation. Animals were housed in the group of 4 per cage at 12 h light-dark cycle (7 am–7 pm), 23 ± 2 °C temperature and 60 ± 5% humidity in the animal house facility of JUIT. Animals had free access to water and were fed with standard mice pellets. All the experimental procedure were performed after approval from the Institute Animal Ethics Committee, in accordance with CPCSEA, India. All precautions were taken to minimize discomfort and suffering to the animals.

2.2. Experimental design

Animals were randomly selected and divided into two groups (n ≥ 16); Group A: Control and Group B: CUS. Both the groups were subdivided into two groups; Group A1: Control (received 0.3% carboxymethyl cellulose as vehicle) (CTRL), Group A2: CTRL + quercetin (CTRL + Q), Group B1: Chronic unpredicted stress (received vehicle) (CUS) and Group B2: CUS + Q. Animals were administered either 30 mg/kg quercetin [8] or vehicle orally for 21 days, once daily, as per the animal grouping. Animals in Group B were subjected to chronic stress for 21 days and stress was given randomly between 8 am to 6 pm as per our previously described method [8]. Stressed animals were housed separately to avoid any sort of contact with the normal animals. Behavioral alterations were evaluated between day 22 and 26 of the study. Animals were sacrificed 24 h after the last behavior test, samples were collected and processed for further molecular studies.

2.3. Locomotor activity and muscle coordination

Actophotometer is a well-established method to evaluate locomotor activity of the rodents [28]. We used this model to investigate the effect of CUS and quercetin treatment on locomotion in mice on day 22 of the study. Apparatus consisted of a chamber (36 × 36 × 10 cm) having light emitting probes on two adjacent walls and photo-detector on the other two walls, such that every time light beam is interrupted, a digital meter records the reading. The movement of animals obstruct the light beam and activity is recorded digitally in term of number of light beam crossed (or number of line crossing). Animals were placed inside the Actophotometer for 10 min experimental session, during which number of line crossings were recorded. Entire apparatus was cleaned with 70% ethanol between every experimental session to eliminate any sign of olfactory cues.

We used Rota-rod apparatus to evaluate the effect of CUS and quercetin treatment on muscle coordination. Animals were placed on the rotating bar of rota-rod apparatus (25 rpm) and time taken by animal to fall from the bar was recorded as a measure of muscle coordination, with the maximum cut-off time of 180 s. Entire apparatus was cleaned with 70% ethanol between every experimental session to eliminate any sign of olfactory cues.

2.4. Depression

Tail suspension test (TST) and forced swim test (FST) are widely used models to evaluate depressive-like behavior in the rodents [28]. We used these models to determine the effect of quercetin treatment on CUS mediated depression. TST was performed by suspending mice 60 cm above the surface of the floor, from the edge of a beam by placing an adhesive tape 5 cm below the base of their tail. In an experimental session of 6 min, frequency of upward turning and total immobility time was recorded as the measure of depression-like behavior. Immobility was considered when all the struggling body movements of the animals ceases and it remain hanging from its tail motionless.

Likewise, FST was performed by forcing animals to swim inside a glass cylinder (50 cm high and 25 cm radius) filled with water up to 25 cm at 23 ± 2 °C. During an experimental session of 6 min, total time spent by animals in immobile posture was recorded as a measure of depression. Animals were considered immobile when all its body moment, except respiration, ceases and it remain floating motionless in the water. Gentle movements needed to keep the head above the surface of water were also recorded as immobility duration.

2.5. Memory

Passive avoidance step down test (PASD) model is used to evaluate memory performance in rodents [28,29]. We used this model to evaluate the effect of quercetin treatment on short term and long term memory retention in CUS challenged animals. Experiment was performed inside a wooden chamber (50 × 50 × 50 cm) lined with grid floor which was capable of delivering inescapable foot shock to the animals. A square wooden platform (5 × 5 cm) was placed in the center of the apparatus. On day 22, learning trial was performed during which animals were placed on the platform and step-down latency was recorded. As soon as all four paws of the animal touches the grid floor, a foot shock (0.2 mA) was delivered through grid floor. Short-term memory was evaluated after 24 h of learning trial. Animals were placed