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Research report

Metformin and ascorbic acid combination therapy ameliorates type 2 diabetes mellitus and comorbid depression in rats

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ABSTRACT

Diabetes mellitus and depression are the common comorbid disorders affecting humans worldwide. There is an unmet need to develop therapeutic strategies to treat both diabetes mellitus and comorbid depression. The present study evaluated the effectiveness of metformin and ascorbic acid against type 2 diabetes mellitus and comorbid depression in rats. Four groups of diabetic rats were orally administered with vehicle (1 mL/kg), metformin (25 mg/kg), ascorbic acid (25 mg/kg), or combination of metformin (25 mg/kg) and ascorbic acid (25 mg/kg) for 11 consecutive days. Diabetes was induced by single-dose administration of streptozotocin (65 mg/kg, i.p.) with nicotinamide (120 mg/kg, i.p.). Comorbid depression was induced by five inescapable foot-shocks (2 mA, 2 ms duration) at 10 s intervals on days 1, 5, 7, and 10. One group of healthy rats received only vehicles to serve as nondiabetic control group. On day 11, animals were sacrificed, and blood and brain samples were collected from each rat following forced swim test. Plasma glucose, insulin, and corticosterone levels were estimated in plasma. The levels of monoamines, proinflammatory cytokines, and oxidative stress were measured in prefrontal cortex. The combination therapy significantly reduced immobility period, glucose, and corticosterone levels relative to diabetes with comorbid depression group. Furthermore, the combination therapy increased the levels of insulin and monoamines, and caused a significant reductions in oxidative stress and proinflammatory cytokines. In conclusion, the present study revealed that metformin and ascorbic acid combination therapy could be a potential strategy to treat type 2 diabetes mellitus and comorbid depression.

1. Introduction

Diabetes mellitus is the most common chronic metabolic disorder with a prevalence of 8.3% globally (Guariguata et al., 2014). According to World Health Organization, diabetes mellitus will become the seventh leading cause of mortality worldwide in 2030 (Mathers and Loncar, 2006). There are mainly three types of diabetes mellitus, which are type 1 (insulin dependent diabetes mellitus), type 2 (non-insulin dependent diabetes mellitus) and gestational diabetes mellitus (hyperglycemia during pregnancy) (AmericanDiabetesAssociation, 2014). According to the global statistics of diabetes mellitus in 2013, about 382 million people are afflicted with diabetes mellitus and type 2 diabetes contributes up to 90% of cases (Tao et al., 2015). A recent report revealed that depression is a common comorbid condition frequently observed in type 2 diabetic patients, one of four patients experiences depressive disorders (Semenkovich et al., 2015). There is a bidirectional relationship between depression and type 2 diabetes mellitus (Stuart and Baune, 2012). A great body of literature supports the fact that depression is a frequent comorbid condition of both type 1 and type 2 diabetes mellitus and share several common pathophysiological mechanisms (Holt et al., 2014; Korczak et al., 2011; Nouwen et al., 2010). The pathophysiology of depression in diabetic patient is multifactorial, which includes functional insulin-resistance, inflammation, oxidative stress, decreased activity of norepinephrine (NE) and serotonin (5-HT), and decreased brain-derived neurotrophic factor (Kai et al., 2000; Krabbe et al., 2007; Lustman and Clouse, 2005; Musselman et al., 2003; Winokur et al., 1988). The comorbid depression increases both micro- and macro-vascular complications, the major cause of multi-organ damage and mortality (Gispen and Biessels, 2000; Semenkovich et al., 2015). Previous preclinical studies evidenced an increased risk of development of depression due to decreased functional activity of central neurotransmitters, such as NE and 5-HT in diabetic rats (Arafa et al., 2016; Haider et al., 2013; Trulson and Himmel, 1985). Inflammation and immune activation have been implicated in the pathogenesis of both diabetes and depression

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(Dantzer et al., 2008; Miller and Raison, 2016). It has been suggested that proinflammatory cytokines such as IL-1β, TNF-α, and IL-6 have the potential to interact with insulin sensitivity and pancreatic β-cell function and induce diabetes (Stuart and Baune, 2012). Depression is associated with overactivation of HPA-axis and overproduction of glucocorticoids leading to aberrant glucose homeostasis (Stuart and Baune, 2012). A recent cross-sectional study has indicated a relationship between inflammation and depression in newly diagnosed diabetic individuals (Herder et al., 2017). Furthermore, vitamin C (ascorbic acid) deficiency (Park et al., 2017) and stressful events (Lloyd et al., 2005) contribute in the development of depressive disorders. Despite vast improvement in our understanding on diabetes comorbid depression, there is an unmet need to develop therapeutic strategies to treat both diabetes mellitus and comorbid depression.

Earlier studies have reported a lower circulating ascorbic acid levels in patients with diabetes mellitus (Donin et al., 2016; Sargeant et al., 2000; Shim et al., 2010; Takahashi et al., 2011; Will and Byers, 1996). Ascorbic acid supplementation has been shown to produce antidiabetic activity (Afkhami-Ardekani and Shojaoddiny-Ardekani, 2007; Dakhale et al., 2011) and antidepressant activity (Binfaré et al., 2009; Iwata et al., 2014). On the other hand, metformin is a potent oral hypoglycemic agent now recommended as the first-line therapy for diabetes mellitus (type 2) (Viollet et al., 2012). In addition to its hypoglycemic activity metformin has been shown to elicit marked antioxidant activity (Ashabi et al., 2015; Esteghamati et al., 2013; Gallo et al., 2005; Hou et al., 2010; Nakhjavani et al., 2011), neuroprotective activity (Adedeji et al., 2014; Nath et al., 2009), and antiepileptic activity (Zhao et al., 2014). The pleiotropic pharmacological activities of metformin and ascorbic acid makes them suitable for the treatment of diabetes mellitus and comorbid depression, which involves a myriad of pathophysiological characteristics. However, till date, no studies have been conducted to assess the therapeutic potential of metformin and ascorbic acid combination treatment against diabetes mellitus and comorbid depression in rat.

Considering all the pathophysiological factors, it can be hypothesized that a combination strategy, which can abrogate hyperglycemia, inflammation, oxidative stress, and imbalance in neurotransmitter levels, would be a possible option for treating diabetes mellitus and comorbid depression. In the present study, we explored the potential benefits of metformin and ascorbic acid combination treatment in a rat model of diabetes mellitus and comorbid depression that primarily focuses on a clinical situation where occurrence of diabetes mellitus leads to depression. Experiments were designed to investigate the effects of combination treatment on the markers of depression (immobility period in forced swim test, plasma corticosterone levels, and adrenal hyperplasia), markers of diabetes mellitus (plasma glucose and insulin levels), brain monoamines (levels of NE and 5-HT in the brain), oxidative stress (lipid peroxidation, superoxide dismutase, and catalase activity in the brain), and inflammatory processes (levels of proinflammatory cytokines TNF-α and IL-6 in the brain).

2. Results

2.1. Effects of metformin, ascorbic acid, or their combination therapy on comorbid depression

The existence of comorbid depression was assessed on day 11 by estimating immobility period (Fig. 1A) through forced swim test, plasma corticosterone levels (Fig. 1B), and adrenal hyperplasia (Fig. 1C) in diabetic rats. The rats with diabetes and comorbid depression showed significantly ($P < 0.05$) higher immobility periods (140.3 ± 4.4 versus 41.2 ± 5.2 s; Fig. 1A) compared with nondiabetic controls. Monotherapy of both metformin (92.7 ± 4.5 versus 140.3 ± 4.4 s; Fig. 1A) and ascorbic acid (109.2 ± 5.7 versus 140.3 ± 4.4 s; Fig. 1A) at 25 mg/kg, p.o. showed significant ($P < 0.05$) decrease in immobility period as compared to the DCD control rats. However, the decrease in immobility period was significantly ($P < 0.05$) higher in metformin monotherapy group compared with ascorbic acid monotherapy group (92.7 ± 4.5 versus 109.2 ± 5.7 s; Fig. 1A). The combination (metformin and ascorbic acid at 25 mg/kg, p.o.) therapy showed an additive synergistic effect, with significant decrease ($P < 0.05$) in immobility period (60.8 ± 4.4 versus 140.3 ± 4.4 s; Fig. 1A) compared with DCD control rats, metformin monotherapy (60.8 ± 4.4 versus 92.7 ± 4.5 s; Fig. 1A) and ascorbic acid monotherapy (109.2 ± 5.7 versus 140.3 ± 4.4 s; Fig. 1A).

Assessment of plasma corticosterone showed significantly ($P < 0.05$) higher levels in rats with diabetes and comorbid depression compared with nondiabetic controls (135.4 ± 3.1 versus 65.4 ± 2.4 ng/mL; Fig. 1B). Monotherapy with metformin (119.0 ± 2.6 versus 135.4 ± 3.1 ng/mL; Fig. 1B) or ascorbic acid (122.8 ± 1.9 versus 135.4 ± 3.1 ng/mL; Fig. 1B) showed a significant ($P < 0.05$) decrease in plasma corticosterone as compared to the DCD control rats. The reduction in plasma corticosterone levels was slightly higher in metformin only treated rats as compared to ascorbic acid only treatment (119.0 ± 2.6 versus 122.8 ± 1.9 ng/mL; Fig. 1B). In line with results of forced swim test, the combination therapy produced an additive synergistic effect, with significant reductions in plasma corticosterone levels compared with DCD controls (111.1 ± 2.6 versus 135.4 ± 3.1 ng/mL; Fig. 1B), metformin monotherapy (111.1 ± 2.6 versus 119.0 ± 2.6 ng/mL; Fig. 1B), and ascorbic acid monotherapy (111.1 ± 2.6 versus 122.8 ± 1.9 ng/mL; Fig. 1B).

In accordance with increase in corticosterone levels, a significant ($P < 0.05$) adrenal hyperplasia was observed in rats with diabetes and comorbid depression compared with nondiabetic control rats (adrenal gland weight 70.4 ± 3.4 versus 44.3 ± 5.8 mg; Fig. 1C). A significant ($P < 0.05$) reduction in adrenal hyperplasia was observed with both metformin (adrenal gland weight 58.7 ± 2.6 versus 70.4 ± 3.4 mg; Fig. 1C) and ascorbic acid (adrenal gland weight 59.6 ± 2.1 versus 70.4 ± 3.4 mg; Fig. 1C) monotherapy relative to the DCD control rats. Monotherapy of metformin and ascorbic acid were equally effective in reducing adrenal hyperplasia (adrenal gland weight 58.7 ± 2.6 versus 59.6 ± 2.1 mg; $P > 0.05$; Fig. 1C). The combination therapy showed significant ($P < 0.05$) reduction in adrenal hyperplasia when compared with DCD control rats (adrenal gland weight 53.9 ± 2.2 versus 70.4 ± 3.4 mg; Fig. 1C). However, the effect observed with combination therapy was statistically insignificant ($P > 0.05$) when compared with metformin monotherapy (adrenal gland weight 53.9 ± 2.2 versus 58.7 ± 2.6 mg; Fig. 1C) and ascorbic acid monotherapy (adrenal gland weight 53.9 ± 2.2 versus 59.6 ± 2.1 mg; Fig. 1C).

2.2. Effects of metformin, ascorbic acid, or their combination therapy on hyperglycemia and insulin levels

Plasma glucose and plasma insulin levels were estimated to assess the antidiabetic efficacy of the combination therapy (metformin and ascorbic acid at 25 mg/kg, p.o.) and monotherapy of both metformin (25 mg/kg, p.o.) and ascorbic acid (25 mg/kg, p.o.) after 11 days of administration. The rats with diabetes and comorbid depression exhibited significant ($P < 0.05$) hyperglycemia (279.5 ± 5.9 versus 93.0 ± 3.6 mg/dL; Fig. 2A) relative to the nondiabetic control rats. Significant ($P < 0.05$) reductions in plasma glucose (140.6 ± 5.2 versus 279.5 ± 5.9 mg/dL; Fig. 2A) were observed in both metformin (156.3 ± 6.7 versus 279.5 ± 5.9 mg/dL; Fig. 2A) and ascorbic acid (236.3 ± 4.9 versus 279.5 ±