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Research report

Effects of metformin on inflammation and short-term memory in streptozotocin-induced diabetic mice

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ABSTRACT

The aim of the present study was to analyze the action of metformin on short-term memory, glial cell activation and neuroinflammation caused by experimental diabetic encephalopathy in C57BL/6 mice. Diabetes was induced by the intraperitoneal injection of a dose of 90 mg/kg of streptozotocin on two successive days. Mice with blood glucose levels ≥200 dl/ml were considered diabetic and were given metformin hydrochloride at doses of 100 mg/kg and 200 mg/kg (by gavage, twice daily) for 21 days. On the final day of treatment, the mice underwent a T-maze test. On the 22nd day of treatment all the animals were anesthetized and euthanized. Diabetic animals treated with metformin had a higher spatial memory score. The hippocampus of the diabetic animals presented reactive gliosis, neuronal loss, NF-kB signaling activation, and high levels of IL-1 and VEGF. In addition, the T-maze test scores of these animals were low. Treatment with metformin reduced the expression of GFAP, Iba-1 (astrocyte and microglial markers) and the inflammation markers (p-IKB, IL-1 and VEGF), while enhancing p-AMPK and eNOS levels and increasing neuronal survival (Fox-1 and NeuN). Treatment with metformin also improved the spatial memory scores of diabetic animals. In conclusion, the present study showed that metformin can significantly reduce neuroinflammation and can decrease the loss of neurons in the hippocampus of diabetic animals, which can subsequently promote improvements in spatial memory.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and the impaired secretion of endogenous insulin, or insulin receptor insensitivity (ROLL, PALM, 2006). According to the World Health Organization (WHO), there were 171 million people with diabetes in the year 2000, and by 2030 this number is expected to reach 366 million (World Health Organization, 2006). Despite this forecast, in 2015 there were already 415 million people with diabetes (International Diabetes Federation, 2015).

This estimate is important because diabetes is related to secondary complications in various organs, related to angiopathic complications (Kowluru and Kennedy, 2001; Kunisaki et al., 1995). Hyperglycemia also causes electrophysiological changes (Allen et al. 2004) and learning and memory impairment (Jolivalt et al., 2008) in the central nervous system (CNS), which is accompanied by neuronal apoptosis (Sima and Li, 2005). Neurodegeneration resulting from hyperglycemia is usually associated with chronic inflammatory responses, generated by reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Mello, 2012).

Hyperglycemia in a culture of endothelial cells induces ROS production, NF-κB activation, increased levels of protein kinase C (PKC) and advanced glycation end products (AGEs). The blockade of ROS generation suppresses the activation of nuclear factor κB (NF-κB), leading to decreased levels of PKC and AGE (Nishikawa et al., 2000). Therefore, the formation of ROS precedes the activation of other systems (Evans et al., 2003).

Vascular complications within the CNS are primarily mediated by AGE and its ability to bind to receptor advanced glycation end (RAGE) products (Chilelli; Burlina; Lapolla, 2013; Niiya et al., 2006, 2012, Brownlee, 2001). While the physiological expression of RAGE is minimal in tissues and vasculature, it is greater in certain

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cell types, such as endothelial and astrocyte, where there is an excess of AGE (Goldin et al., 2006; Toth; Martinez; Zochodne, 2007). AGE-RAGE binding increases oxidative stress and inflammation mediated by NF-kB (Haslbeck et al., 2004). NF-κB activation, which can be triggered by both RAGE and VEGF, also increases VEGF expression, leading to a vicious cycle (Evans et al., 2003). In diabetic RAGE-null mice, no NF-κβ activation or endothelial changes have been detected (Myint et al., 2006; Shoji et al., 2006).

In the hippocampus of diabetic mice, an inflammatory response occurs alongside astrocyte and microglial activation and increased GFAP and S100B expression, which possibly influences or exaggerates the process of neuronal death (Nagayach; Patro; Patro, 2014; Nardin et al., 2007). Oxidative stress causes hippocampal neuronal death, as well as a reduction in oligodendrocytes, with a consequent loss of white matter (Francis et al., 2008; Toth et al., 2006). Many studies have shown a reduction in synaptic proteins such as synaptophysin and synapsin 1 (Duarte et al., 2012; Arnold et al., 2014), nerve growth factor (NGF) (Sima and Li, 2005), and learning and memory damage as a result of chronic hyperglycemia (Jolivalt et al., 2008; Sima et al., 2009; Alvarez et al., 2009). Inevitably, these changes lead to synaptic communication disorders.

A number of studies have identifies the beneficial effects of metformin, which protects the peripheral vasculature by reducing inflammation induced by NF-κβ activation, as well as benefiting endothelial dysfunction and protecting the peripheral endothelium via the activation of adenosine monophosphate-activated protein kinase AMPK (Correia et al., 2008; Davis et al., 2006; Majithiya and Balaraman, 2006). However, it is not possible to generalize the data obtained from the peripheral vessels of the CNS, due to the many differences between such vessels and the brain blood vessels (Ge; Song; Pachter, 2005).

The aim of the present study was to analyze the action of metformin on short-term memory, glial cell activation and neuroinflammation in experimental diabetic encephalopathy in C57BL/6 mice.

2. Results

2.1. Glycaemia and body weight

Fasting glycaemia was measured on day 22 of diabetes induction and 4 h after metformin administration. There was no significant difference between the glucose levels of the control group (107.4 mg/dL ± 18.10) and the group that received 200 mg/kg of metformin (104.2 mg/dL ± 15.4). However, there were significant differences ($p < 0.05$) between the STZ (347.75 mg/dL ± 52.39), STZ+M100 (321.5 ± 39.26), STZ+M200 (333.75 mg/dL ± 56.09) groups and the control groups (control and M200) (Fig. 1). The same results were observed in all animals after 8 h and overnight fasting (data not shown). The average body weight of the STZ (28.85 ± 0.93), STZ+M100 (22.40 ± 2.20) and STZ+M200 (22.50 ± 2.32) groups at the end of the experiment was significantly lower ($p < 0.05$) than the control group (28.22 ± 0.93) and the M200 group (27.00 ± 3.30) (Fig. 2).

2.2. Metformin improves memory, especially in diabetic animals

The diabetic animals had a significantly lower T-maze score than the animals from the control groups ($p < 0.05$). Treatment with 100 mg/kg of metformin did not improve the performance of the diabetic animals. However, there was a significant increase in the spatial memory score ($p < 0.05$) of diabetic animals that received 200 mg/kg of Metformin. The non-diabetic animals that received 200 mg/Kg of metformin had similar results to the control group (Fig. 3).

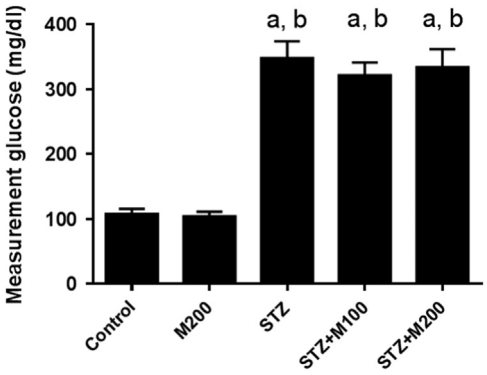

Fig. 1. Effects of metformin on glycemic control. Levels of glycaemia (mean ± S.D) 4 h after metformin was administered using analysis variance (ANOVA), post-hoc Tukey test. [a]$p < 0.05$ when compared to control group, [b]$p < 0.05$ when compared with M200 group, [c]$p < 0.05$ when compared to STZ group, [d]$p < 0.05$ when compared to STZ+M100 group and [e]$p < 0.05$ when compared to STZ+M200 group.

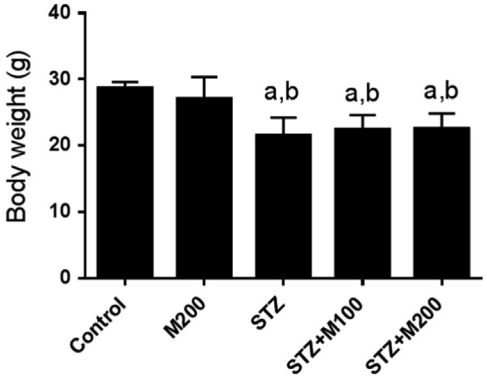

Fig. 2. Effects of metformin on body weight (mean ± S.D) at end of study, using analysis variance (ANOVA), post-hoc Tukey test. [a]$p < 0.05$ when compared to control group, [b]$p < 0.05$ when compared to M200 group, [c]$p < 0.05$ when compared to STZ group, [d]$p < 0.05$ when compared to STZ+M100 group e [e]$p < 0.05$ when compared to STZ+M200 group.

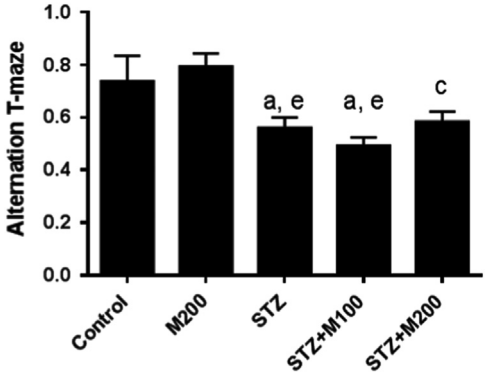

Fig. 3. Effects of metformin on memory. Analysis of alternation on T-maze (mean ± S.D), using analysis variance (ANOVA), post-hoc Dunnet test. [a]$p < 0.05$ when compared to control group, [b]$p < 0.05$ when compared to MET200 group, [c] $p < 0.05$ when compared to STZ group, [d]$p < 0.05$ when compared to STZ+M100 group and [e]$p < 0.05$ when compared to STZ+M200 group.

2.3. Metformin ameliorated neuron survival in the dentate gyrus in mice with diabetes induced by STZ

Immunohistochemistry analysis for FOX-1 revealed a constitutive stain on the mature pyramidal neurons located in the molecular area (arrow) and on the granulated neurons in the dentate gyrus (asterisk) of control group animals (Fig. 4A). The non-diabetic animals that received 200 mg/Kg of metformin